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THE ACUTE AND CHRONIC TOXICITY OF ISOPROPYL ALCOHOL*

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THE similarity in physical properties of isopropyl and ethyl alcohol has suggested the substitution of the former for the latter. Although not a complete substitute in every instance, isopropyl alcohol has found considerable use in industry. Industrial toxicology of the alcohol as the result of occupational exposure apparently has not been a serious problem. However, with the present acute shortage of ethyl alcohol, a wider application of isopropyl alcohol is in prospect, especially for cosmetic and hygienic purposes. For this reason its acute and chronic effects assume a greater importance than heretofore.

Isopropyl alcohol has been of considerable scientific interest in the past, and several summaries of the literature have appeared, notably those of Munch and Schwartze[1] and Morris and Lightbody.[2] Most of the published reports have been confined to acute effects as observed in various animals, special organs, and microorganisms, using a variety of methods. In general, these investigations agree that isopropyl alcohol is approximately twice as active in its toxic propensities as ethyl alcohol.

Studies on chronic intoxication have not been very extensive. Pohl[3] administered orally a total of 224 c.c. of isopropyl alcohol in small daily doses during a period of two months to a puppy and found that the alcoholized animal showed a greater gain in weight than an untreated litter mate. Boruttau[4] in a similar experiment gave 5 c.c. per kilogram for several weeks to one dog and observed no harmful effects. Morris and Lightbody[2] reported experiments

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on rabbits, the object of which was to determine the development of tolerance. They found a decreased rate of recovery from the narcotic effects during the last four days as compared with the first four days of an eleven-day observation period. This was ascribed by them to an accumulation of alcohol, because of slow destruction and elimination, and to the production of a depressant intermediary metabolite, probably acetone.

Much of the earlier data are subject to considerable variation, probably the result of using an alcohol of doubtful purity[3] as well as the employment of different doses and methods of administration. In view of this fact and also in anticipation of projected experiments on absorption, combustion, and distribution of isopropyl alcohol, it seemed necessary to re-establish minimum lethal doses with a product of known purity and with adherence to a standard technique. Therefore, we undertook a comprehensive study of acute and chronic toxicity of isopropyl alcohol, employing a product at least 99 per cent pure. Some comparisons with ethyl alcohol have also been made.

HEMOLYTIC ACTION

Based on the evidence[2] that isopropyl alcohol is slowly destroyed and eliminated, suggesting a prolonged sojourn in the blood stream, hemolysis of blood cells and precipitation of blood proteins may occur. This continued injury, should it develop, could be responsible for the reported delayed deaths in animals. The importance of the blood effects also becomes apparent in metabolic studies where prompt and complete absorption of any specific dose must be assured and intravenous administration is the method of choice. Hemolytic actions were observed in vitro, using a technique similar to that employed in testing red cell fragility. One cubic centimeter of whole rabbit blood was added to a series of tubes containing isopropyl alcohol in concentrations ranging from 0 to 100 per cent in 10 per cent increments, with and without sodium chloride. All tubes were inspected at the end of three and twenty-four hours. Isopropyl alcohol in water produced immediate hemolysis in 10 and 20 per cent concentrations. Dilutions prepared with 0.9 per cent sodium chloride offered complete protection in the 10 per cent tube, but produced complete hemolysis in the 20 per cent tube at the end of three hours. This result was not significantly altered when final concentrations of sodium chloride in each tube were maintained at 0.9 per cent. Concentrations of isopropyl alcohol above 20 per cent regardless of the diluent produced precipitation of blood proteins, the amounts of precipitate varying directly as the alcoholic concentrations increased. It appears that exposure of blood to 10 per cent isopropyl alcohol in 0.9 per cent sodium chloride is a condition which if it obtained within the circulatory system, would not produce any grossly detectable injury to this tissue. This is at least twenty times the blood alcohol concentrations which could be reached with even a fatal dose, and delayed effects of such exposures would not seem to be a contributory cause of death.

INTRAVENOUS TOXICITY

This was studied in ten rabbits and seven dogs which received isopropyl alcohol by slow infusion into a suitable vein, a 20 per cent solution in 0.9 per cent sodium chloride being given at the rate of 0.25 c.c. per kilogram per min-

ute. This slow infusion rate could not be maintained in rats, and after ten trials with widely varying results no further attempts were made. The anesthetic dose was also determined as the amount necessary to produce elimination of the corneal reflex. Table I shows the average anesthetic and fatal doses for the alcohol in rabbits and dogs.

TABLE I

AVERAGE ANESTHETIC AND FATAL DOSES OF ISOPROPYL ALCOHOL ACCORDING TO INTRAVENOUS INFUSION IN RABBITS AND DOGS

ANIMAL	NUMBER OF EXPERIMENTS	ANESTHETIC DOSE C.C. PER KILOGRAM	FATAL DOSE C.C. PER KILOGRAM
Rabbit	10	3.23	8.23
Dog	7	3.35*	5.12

*An average for five dogs.

It is seen that the anesthetic dose for both species is almost identical, but represents only 39 per cent of the fatal dose for rabbits as against 65 per cent of the fatal dose for dogs.

GASTRIC TOXICITY

Isopropyl alcohol was administered gastrically to one hundred and forty white rats, thirty-five rabbits, and one hundred and two dogs. Food was withdrawn from all animals for twenty-four hours before administration. Suitable sounds or stomach tubes were employed, the alcohol being introduced as a 50 per cent solution in 0.9 per cent sodium chloride or water in rats and rabbits. Dogs did not tolerate the alcohol well. After some preliminary trials it was found that a 25 per cent concentration in warm tap water was the most suitable. Vomiting occurred in sixty-two dogs and these animals were discarded. A period of three days was allowed for a fatal outcome. The results are shown in Table II.

TABLE II

TOXICITY OF ISOPROPYL ALCOHOL ACCORDING TO GASTRIC ADMINISTRATION IN RATS, RABBITS, AND DOGS

DOSE C.C. PER KG.	WHITE RATS		RABBITS		DOGS*	
	NUMBER OF ANIMALS USED	PER CENT MORTALITY	NUMBER OF ANIMALS USED	PER CENT MORTALITY	NUMBER OF ANIMALS USED	PER CENT MORTALITY
8.0	10	100			1	100
7.5	20	75	5	100	3 (2)	100
7.0	25	44	5	100	6 (4)	67
6.5	25	56	10	90	9 (5)	55
6.33			10	0		
6.17			5	0		
6.0	20	25			8 (9)	37
5.5	15	13			5 (23)	60
5.0	15	7			6 (8)	33
4.5					2 (11)	0
2.5	10	0				

*Emesis was commonly exhibited in dogs, and the number discarded therefore on each dose appears in parentheses.

The surely fatal dose in white rats was 8.0 c.c. per kilogram; in rabbits 7.0 c.c. per kilogram; and in dogs 7.5 c.c. per kilogram. The 50 per cent killing dose for each species, as calculated by the method of Reed and Muench,[6] was 6.73 c.c., 6.41 c.c., and 6.15 c.c. per kilogram or 84 per cent, 91 per cent, and 82 per cent of the surely fatal dose for rats, rabbits, and dogs, respectively.

CHRONIC TOXICITY

The cumulative effects of isopropyl alcohol in animals might be reflected in growth and body-weight changes after imbibition of the alcohol for prolonged periods. Intermediate oxidation products or the alcohol as such could be responsible for tissue damage. For a study of the effects of chronic intoxication the procedure of voluntary drinking of isopropyl alcohol by white rats was employed.

Seven groups of five rats each of about 50 grams body weight were placed in cages and given free access to Steenbach's whole artificial diet.* Two groups, one of males and another of females, were given water to drink and thus served as controls. The other groups were given 0.5 (males), 1.0 (females), 2.5 (males), 5.0 (females), and 10.0 (males) per cent solutions of isopropyl alcohol in water. The rats were weighed weekly, the food weighed as often as necessary, and fluid intake was recorded daily. This regimen was maintained for twenty-seven weeks, and when the experiment was terminated, all survivors were sacrificed with the exception of three females from each of the control, 1.0 and 5 per cent groups; the latter were used to study the effects of isopropyl alcohol on reproductive functions. Tissues from the sacrificed animals were submitted to Dr. Mark E. Maun of the Department of Pathology, for pathologic study. The essential experimental data are presented as curves in Fig. 1, and show daily changes in body weight, daily food consumption, daily intake of fluid, and daily dose of isopropyl alcohol for all rats from week to week except those drinking 10 per cent alcohol.

The results were as follows: the rats which received 10 per cent isopropyl alcohol refused to drink this fluid and died in from seven to twenty-eight days; hence the results were discarded. Female rats drinking 1 per cent and 5 per cent alcohol remained well throughout the observation period. In the 0.5 per cent group one animal died during the fifth week, and a second rat succumbed the sixth week. In the 2.5 per cent group, deaths occurred in the eighth, twentieth, and twenty-second week. Whether these deaths were due to natural causes or to a greater susceptibility of the male rat to isopropyl alcohol could not be ascertained, as the rats were found dead unexpectedly, and extensive post-mortem changes precluded an accurate diagnosis by the pathologist.

Growth and body weight curves of the surviving males in each group showed some retardation during the first thirteen weeks of observation, or about 5 per cent and 17 per cent, respectively. A definite gain was noted in the last fourteen weeks. Actually weights were 5.6 per cent greater for the 0.5 per cent group and 9.1 per cent greater for the 2.5 per cent group than those of the controls at the end of twenty-seven weeks. Since it is known that isopropyl alcohol is burned in the body, the increased caloric intake may, in part at least, account for these increases. Female rats on 1 per cent and 5 per cent alcohol showed some depression in growth and body weight, which at the termination of the experiment amounted to a 12 per cent loss in the former group and a 10.3 per cent loss in the latter group. Food intake in all rats showed that appetite was not impaired, the quantity consumed by the test animals being equal to or

*This diet contained: feed corn meal, 68 parts; linseed oil cake meal, 10 parts; dried ground alfalfa, 2 parts; powdered casein, 10 parts; Crisco, 5 parts; cod liver oil, 3 parts; bone ash, 1.5 parts; and sodium chloride, 0.5 part.

greater than that of the control animals. There was a tendency for a decreased fluid intake as alcoholic concentrations increased, which is to be attributed partially to the unpalatability and partially to the depressant action of the alcohol.

The amounts of isopropyl alcohol ingested were quite large, the daily intake averaging about 1/11, 1/6, 3/8, and 5/8 of the oral, surely fatal, dose in ascending order of concentration. Careful notes were made on the behavior of the alcoholized rats, but aside from the slight retardation of growth and probable accidental death of some rats in the 0.5 per cent and 2.5 per cent groups, no

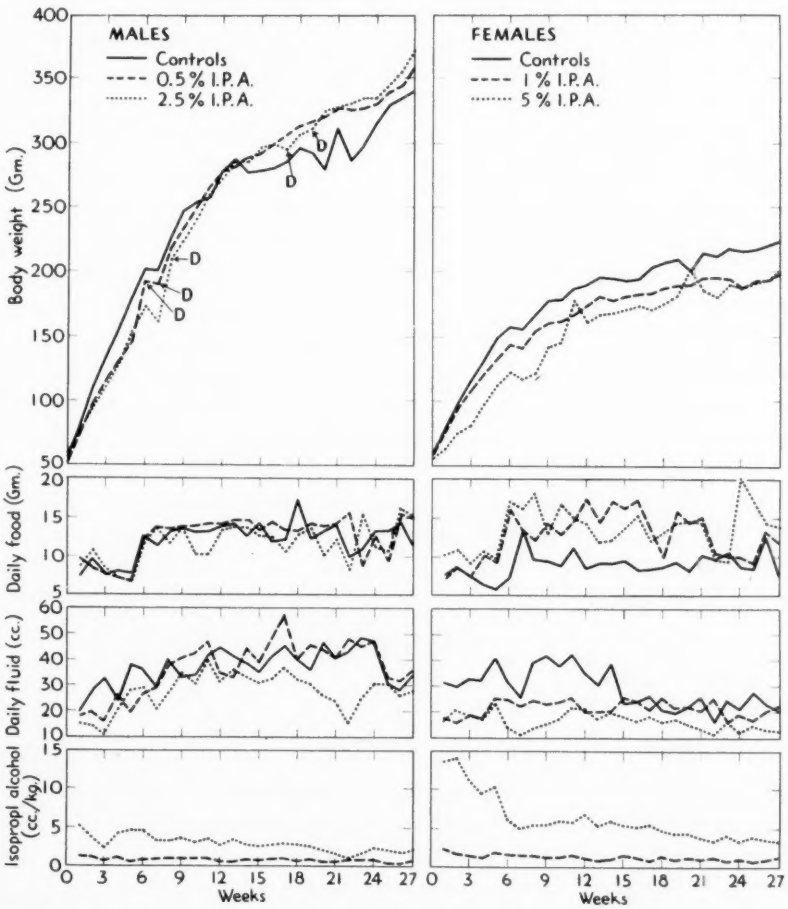

Fig. 1.—Effect of continued drinking of isopropyl alcohol upon white rats. *D* represents the death of one animal at the time. *I.P.A.* = Isopropyl alcohol.

definite manifestations of poisoning were observed. Gross and microscopic examinations of the brain, pituitary, lung, heart, liver, spleen, kidneys, and adrenals showed no evidence of gross or pathologic changes. It would seem, therefore, that retardation of growth and body weight loss were general physiologic effects rather than reflections of injury to some specific function or organ.

COMPARATIVE TOXICITY

Correlation of the toxicity of isopropyl and ethyl alcohols was established in rats, rabbits, and dogs. These were limited to the oral route since there is no

known indication for the use of isopropyl alcohol intravenously, and the more important aspects are associated with gastric ingestion. The 50 per cent fatal dose was established for ethyl alcohol in fifty rats, thirty-five dogs, and sixteen rabbits; however, because of the similarity of the results of our experiments with those of Morris and Lightbody,[2] the more expansive data of the latter investigators were utilized to calculate the M.L.D. 50 dose in rabbits. A summary is presented in Table III.

TABLE III

COMPARATIVE TOXICITY OF ISOPROPYL AND ETHYL ALCOHOL ACCORDING TO ORAL ADMINISTRATION IN RATS, RABBITS, AND DOGS

ANIMAL	M.L.D. 50 IN C.C. PER KILOGRAM		RATIO
	ISOPROPYL	ETHYL	ETHYL = 1.0
Rat	6.73	12.96	1.92
Rabbit	6.41	10.22	1.56
Dog	6.15	12.25	1.99

The results are in agreement with those obtained by other investigators and indicate that isopropyl alcohol is approximately twice as toxic as ethyl alcohol. If this relationship holds for man, the surely fatal dose of isopropyl alcohol may be calculated from the data supplied by Jetter.[7] He reported two confirmed deaths from acute alcoholism. Blood alcohol concentrations were 480 mg. per 100 c.c. and 470 mg. per 100 c.c., and, according to Newman,[8] this represented the rapid imbibition of about one and a half pints of whiskey. It follows that approximately 166 c.c. of isopropyl alcohol could be a fatal dose for man. When the emetic action of the alcohol is taken into consideration, it is extremely doubtful whether this quantity would be tolerated as a single dose. Furthermore, depression of the central nervous system, which appears with a dose considerably below the fatal dose as observed in animals, would limit ingestion, and the combination of the emetic and depressant actions would definitely lessen the probability of ingesting a fatal quantity.

COMMENT

Throughout the course of our experimental work the general effects of isopropyl alcohol followed closely the sequence of toxic actions of ethyl alcohol. The actions of significant doses upon the central nervous system were depression and later paralysis. Although nystagmus was repeatedly observed, other ocular symptoms such as impairment of vision as determined subjectively were not seen in the several hundred animals used. Salivation, retching, and vomiting were somewhat less frequent in the ethyl alcohol series of dogs than in the isopropyl alcohol series; actually, an incidence of 48.3 per cent for the former and 60.7 per cent for the latter. Vomiting in the isopropyl alcohol series occurred at two stages of intoxication; early, or within five minutes after administration of the alcohol and which was undoubtedly due to distention and irritation, and later, after absorption had progressed to the coma state but with the corneal reflex still present. This delayed effect was attributed to a central action, since vomiting was observed in two dogs which received intravenous toxic doses, and retching was a rather frequent occurrence. These observations do not differ from those of ethyl alcohol as reported by Newman in his review already referred to.

Delayed toxic effects as suggested by Morris and Lightbody have not been observed. All of the fatalities among the animals to which the alcohol was administered orally occurred within twenty-four to thirty-six hours, and if any animal showed signs of recovery before this time interval, complete return to normal insofar as appetite and behavior were concerned was assured. To test the matter further, two dogs were given one-half, and three dogs three-fourths, of the intravenous fatal dose and allowed to recover. Within twenty-four hours no symptoms of intoxication were apparent and the animals remained well. The rather slight effects on growth and body weight in rats in chronic intoxication would also seem to rule against any delayed toxic action.

CONCLUSIONS

1. Isopropyl alcohol, as determined in several species of animals after various routes of administration, is approximately twice as lethal as ethyl alcohol.

2. The continuous consumption of isopropyl alcohol in doses of 0.75 to 5.28 c.c. per kilogram per day may either augment growth and body weight in rats or produce a slight decrease.

3. Evidence of delayed toxic effects was not observed, and the results of chronic intoxication would seem to argue against the production of a harmful intermediary metabolite.

4. The effects of isopropyl alcohol in animals are similar to those of ethyl alcohol. Salivation, retching, and vomiting appear more frequently, but the general sequence of terminal symptoms proceeding to paralysis is similar to that elicited by ethyl alcohol.

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THE TREATMENT OF PNEUMOCOCCAL PNEUMONIA WITH SULFAMETHAZINE*

Preliminary Report

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SULFAMETHAZINE, 2-(4′-aminobenzene-sulfonylamino 4:6-dimethylpyrimidine), was first used in the treatment of pneumonia by Macartney et al., in Manchester, England.[1] Subsequently, Jennings,[2] also in England, reported the treatment of pneumonia with this drug in infants and children. These workers found that sulfamethazine, as well as being therapeutically effective, caused very few toxic reactions.

Rose, Martin, and Bevan[3] reported the results of studies on the chemical, pharmacologic, and therapeutic properties of sulfamethazine. Comparing sulfamethazine with sulfapyridine, they found that sulfamethazine, administered over a period of two weeks to albino rats, did not produce renal damage, while sulfapyridine, similarly administered, produced pathologic changes in the kidneys like those caused by sulfathiazole. In human beings, despite the fact that high blood concentrations were obtained clinically, crystalluria and hematuria never occurred. This was attributed to the fact that both sulfamethazine and acetyl sulfamethazine were highly soluble in Ph range of 5.5 to 8 (normal Ph range of urine).

We reported recently[4] the results of a comparative study on the treatment of Type I pneumococcal infections in albino rats in which sulfathiazole, sulfadiazine, and sulfamethazine were used alone or in combination with Type I antipneumococcal serum. We found that sulfamethazine alone, and when combined with serum, produced respectively higher survival rates than any of the other sulfonamides similarly employed.

It is the purpose of this paper to present and analyze the results of treatment with sulfamethazine in forty-eight cases of pneumococcal pneumonia.

Plan of Study.—During the three months period, from March 1 to May 31, 1943, forty-eight adult patients (over 15 years of age), with pneumococcal pneumonia, were admitted to the medical services of the Long Island College and Kings County Hospitals in Brooklyn. In each of these cases, isolation of pneumococci from the sputum was accomplished, and together with a typical

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The sulfamethazine used in this study was donated by the Nepera Chemical Company.

symptomatology, physical and roentgenographic findings established the diagnosis of pneumococcal pneumonia. Treatment, however, in no case was delayed until the causative organism was identified, but was begun as soon as the existence of pneumonia had been clinically determined.

The pneumococci were identified by direct Neufeld typing which was confirmed by the typing of the peritoneal exudate from mice inoculated with the sputum. Cultures of the blood were made upon admission to the hospital and were repeated daily during the febrile period. Blood studies, including red and white blood cell counts, hemoglobin, and urea nitrogen determinations were made at least every other day. Daily examinations of the urine were made for the presence of crystals of sulfamethazine and acetyl sulfamethazine. Determinations of both free and total concentrations of sulfamethazine in the circulating blood were made at daily intervals.

AVERAGE FREE AND TOTAL BLOOD SULFAMETHAZINE LEVELS AND AVERAGE PERCENTAGE OF CONJUGATION IN PNEUMOCOCCAL PNEUMONIA

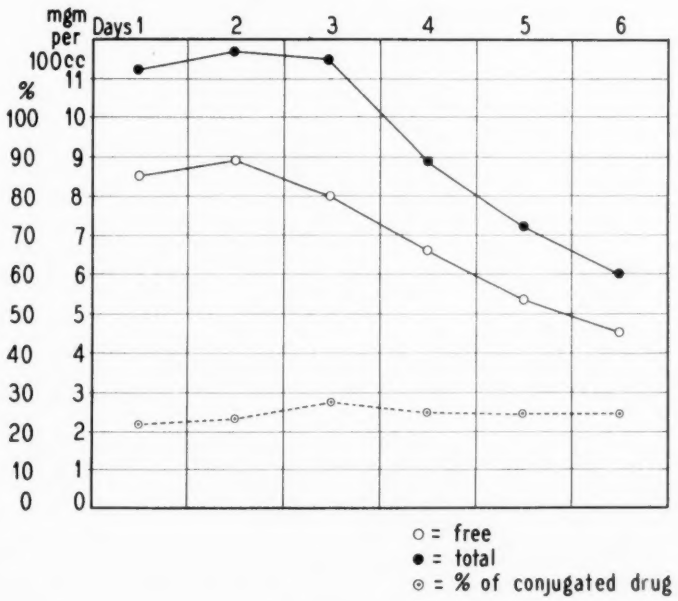

Chart 1.

Dosage of Sulfamethazine.—All the patients were given an initial dose of four grams orally followed by one gram every four hours day and night until the temperature was normal for approximately sixty hours. With this dosage, adequate blood levels were usually obtained. On the first two days of treatment, free sulfamethazine in the blood averaged 8.76 mg. per 100 c.c. with extremes of 1.3 and 16.0 mg. per 100 c.c. The average degree of conjugation during the first six-day period, on the last day of which 29 per cent of the patients were

represented, averaged 24.0 per cent, a peak of 25.7 per cent having been reached on the fourth day (Chart I), with extremes of 0 per cent and 55 per cent (Chart II).

Analysis of Cases (Table I).—Nineteen types of pneumococci were found in the sputum or sputum and blood of these forty-eight cases. Type III pneumonia, occurring in six cases, was the most frequently encountered. There were four cases each of Types II, VII, VIII, and "x," and three each of I, V, and XVIII. Bacteremia occurred in 10, or 20.8 per cent of the cases, there being two each of Types I and II, and one each of Types III, IV, V, IX, XII, and XIV.

Antecedent diseases of significant degrees of severity were encountered in twenty-two cases (Table I). The ages of thirty-eight patients ranged between the fourth and seventh decades inclusively (Table II). The duration of illness and lobar distribution of the pneumonia are shown in Tables III and IV.

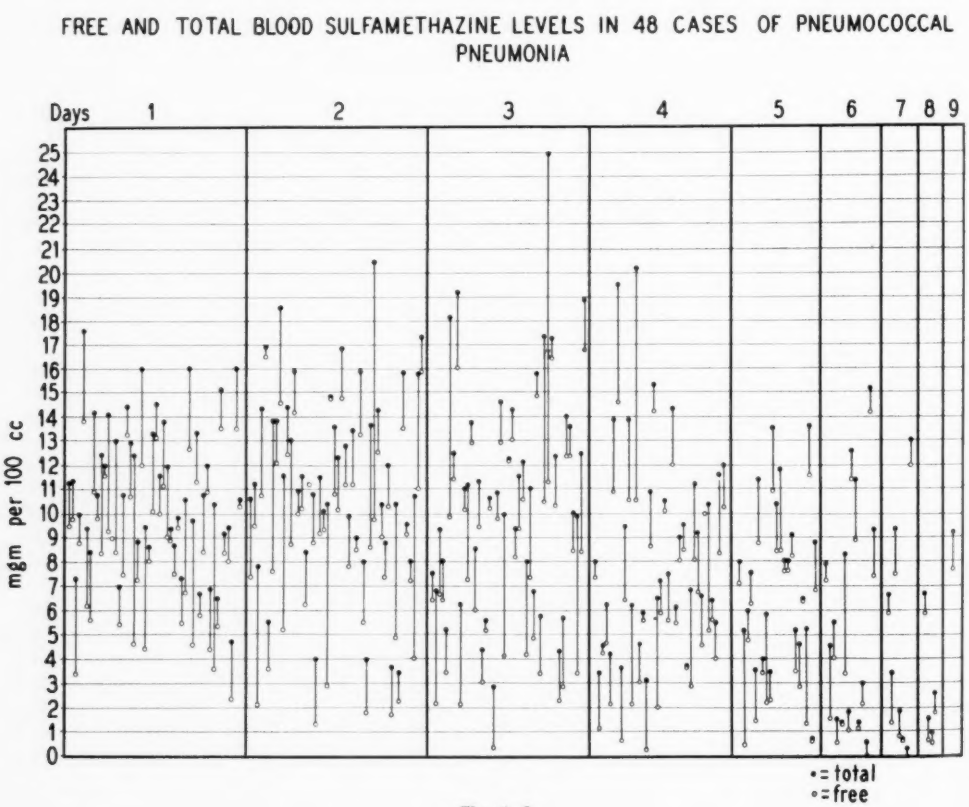

Chart 2.

Results of Treatment (Table I).—All of the patients, except three in the sixth, seventh, and eighth decades respectively, recovered. Each of these three fatal cases had bacteremia and severe antecedent disease conditions to which apparently the pneumonia was a secondary and terminal condition. One, a 75-year-old white man, with Type I pneumonic involvement of the right middle lobe, had generalized arteriosclerosis and uremia and had been ill for six days before institution of therapy. Another, a 59-year-old white man, with chronic

alcoholism and delirium tremens, and Type XII pneumonia of the right middle lobe, had been ill for two weeks prior to admission. The third, a 65-year-old white man, with Type XIV pneumonia of the right lower lobe, had been ill for two weeks before admission, and had, in addition, arteriosclerotic and hyper-

TABLE I

ANALYSIS OF CASES

TYPE	NO.	BACTEREMIA	ANTECEDENT DISEASES	COMPLICATIONS	TOXIC REACTIONS	DEATHS
I	3	2	Rheum. H. D.		Drug Fever(1)	
II	4	2	Tuberculosis Senility, Malnutrit. Arteriosclerosis Uremia			1(B)
III	6	1	Chr. Leucemia Tuberculosis Diabetes Mellitus	St. Effusion	Drug Fever(1)	
IV	2	1				
V	3	1	Tuberculosis			
VI	2		Bronchiectasis			
VII	4		Rheum. H. D.(2) Art. Scl. H. D.	St. Effusion		
VIII	4		Carcinoma Bronchiectasis Syphilis	St. Effusion(2)		
IX	1	1		St. Effusion		
X	2		Tuberculosis Art. Scl. H. D.			
XI	1		Tuberculosis			
XII	2	1	Chr. Alc; D. T. Art. Scl. H. D. Uremia			1(B)
XIV	1	1	Art. Scl., Hyper. H. D. Cong. Failure Pulmonary Edema			1(B)
XVIII	3		Hypertension			
XIX	1			St. Effusion		
XXIV	2		Chr. Alc; Pulm. Tbc. Diabetes Mellitus			
XXXII	1					
XXXIII	2					
"x"	4					
Total	48	10			2	3
Per cent		20.8			4.2 per cent	

Fatality Rate: Gross 6.3 per cent.
Corrected 0 per cent.
Fatality Rate: Nonbacteremic 0 per cent.
Fatality Rate: Bacteremic: Gross 30 per cent.
Corrected 0 per cent.

TABLE II

AGE DISTRIBUTION OF PATIENTS

AGE (YEARS)	NO. OF PTS.	DEATHS
10-19	2	
20-29	5	
30-39	12	
40-49	13	
50-59	5	1
60-69	8	1
70-70	3	1
	48	3

tensive heart disease and congestive failure; death was attributed to acute pulmonary edema.

The gross fatality rate was 6.5 per cent; the nonbacteremic fatality rate, 0 per cent; the gross bacteremic fatality rate, 30 per cent. The gross fatality rate can be corrected to 0 per cent if the above three cases in which the pneumonia was a terminal affair are excluded.

The clinical response was usually fairly rapid. The temperature fell to, and remained normal in an average of 55 hours, when three patients having febrile periods of 120, 136, and 216 hours (the last, a case of pulmonary tuberculosis) were excluded.

TABLE III

DURATION OF ILLNESS BEFORE INSTITUTION OF THERAPY

NO. OF DAYS	NO. OF PATIENTS	DEATHS
1	8	
2	4	
3	4	
4	1	
5	0	
6	4	1
7	7	1
8 plus	12	1
Undetermined	8	
	48	3

TABLE IV

LOBAR DISTRIBUTION OF PNEUMONIC INVOLVEMENT

LOBES	NUMBER OF PATIENTS	DEATHS
Right upper	3	
Right middle	2	2
Right lower	19	1
Left lower	17	
Left upper	0	
Multilobar		
Right	2	
Left	1	
Bilateral	4	
Total	48	3

The blood cultures in those patients with bacteremia were rendered sterile within twenty-four hours after beginning sulfamethazine therapy.

There were no purulent pneumococcal complications, and the only complications encountered were six instances of sterile pleural effusion.

Drug Toxicity.—The only toxic reactions observed were two instances of drug fever. Nausea and vomiting did not occur. Crystalluria and hematuria were never encountered, and none of the patients developed any increase in blood urea nitrogen. In two cases, the white blood cells dropped temporarily on the second day of treatment from 8,700 and 5,000 to 3,900 and 2,100 per cm. respectively at the time of crisis. There were no dermatologic nor neurologic reactions.

SUMMARY

The results of treatment with sulfamethazine in forty-eight cases of pneumococcal pneumonia are presented.

The gross fatality rate in this group of cases, of which 20.8 per cent had bacteremia, was 6.2 per cent; the corrected fatality rate was 0 per cent.

The only toxic effects noted were two instances of drug fever. There were no instances of crystalluria nor hematuria despite the fact that there were high concentrations of sulfamethazine in the blood.

The members of the staffs of the hospitals named, notably Doctors Tasker Howard, George H. Roberts, Henry Wolfer, and Joseph G. Terrence, permitted us to treat patients on their services with sulfamethazine.

We should like to express our appreciation to Doctors Janet Watson, Henry Chin, Benjamin C. Berliner, Julius Wolfe, Herbert Bermont, and Jacob Halpern for their clinical assistance, and to Miss Helen Sage for her technical assistance. In addition, we wish to thank Sidney M. Karlton for his cooperation throughout this study.

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THE PATHOLOGIC CHANGES PRODUCED BY PROLONGED ADMINISTRATION OF SULFAPYRAZINE AND SULFAMETHYLDIAZINE (SULFAMERAZINE) IN THE KIDNEYS OF RABBITS AS COMPARED WITH SULFATHIAZOLE AND SULFADIAZINE*

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THE occurrence of severe damage of the kidneys after prolonged administration of sulfonamides makes it necessary to subject this unwelcome symptom to special investigation. In many cases clinical observation and autopsies have revealed the pathogenesis and the site of injury in the urinary organs observed after administration of the various sulfonamide compounds. In addition, animal experimentation has given ample opportunity for comparing, at a given dosage schedule, the nephrotoxic effect of the compounds and for studying the pathologic tissue changes produced by prolonged drug administration.

A great many of the sulfonamide derivatives now in common use and several new products have been tested in this respect.[1-10] Judging from the literature, however, there exist differences of opinion concerning the following questions: (1) which derivative offers the highest chemotherapeutic activity associated with the highest possible security against kidney injury; (2) which doses and which period of daily administration are sufficient with the various derivatives to produce pathologic changes in the urinary organs; (3) which derivative has shown the greatest ability for the formation of gross concrements (uroliths) in the urinary passages?

In particular, the occasional observation of renal complications associated with the clinical use of sulfathiazole and sulfadiazine, such as hematuria, uroliths, suppression of the urinary output, stimulated the research for new derivatives of an equal therapeutic but a less nephrotoxic activity. For this reason especially, the para-isomer of sulfadiazine (sulfapyrazine) and both the dimethyl derivative of sulfadiazine (sulfamethazine, sulfamethylthiazole) and the monomethyl derivative (sulfamerazine, sulfamethyldiazine) were submitted to experimental investigation.

In our experimental study we were concerned with determination of the extent and site of pathologic changes produced in the kidneys of rabbits by prolonged oral administration of sulfapyrazine and sulfamethyldiazine (sulfamerazine) as compared with sulfathiazole and sulfadiazine.

EXPERIMENTAL

Forty-eight rabbits of 1800 to 2100 grams of weight were divided into four groups of 12 animals each. Each group was treated with one of the four compounds. The compounds were administered orally in capsules twice a day for

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ten days in succession (20 doses). The doses were given at 10 A.M. and 3 P.M. All compounds were administered in 4 different doses as follows:

I. Group
- 3 rabbits treated with sulfathiazole 0.05 per 1000 Gm. of body weight
- 3 rabbits treated with sulfathiazole 0.1 per 1000 Gm. of body weight
- 3 rabbits treated with sulfathiazole 0.2 per 1000 Gm. of body weight
- 3 rabbits treated with sulfathiazole 0.25 per 1000 Gm. of body weight

II. Group
- 3 rabbits treated with sulfamerazine 0.05 per 1000 Gm. of body weight
- 3 rabbits treated with sulfamerazine 0.1 per 1000 Gm. of body weight
- 3 rabbits treated with sulfamerazine 0.2 per 1000 Gm. of body weight
- 3 rabbits treated with sulfamerazine 0.25 per 1000 Gm. of body weight

III. Group
- 3 rabbits treated with sulfapyrazine 0.05 per 1000 Gm. of body weight
- 3 rabbits treated with sulfapyrazine 0.1 per 1000 Gm. of body weight
- 3 rabbits treated with sulfapyrazine 0.2 per 1000 Gm. of body weight
- 3 rabbits treated with sulfapyrazine 0.25 per 1000 Gm. of body weight

IV. Group
- 3 rabbits treated with sulfadiazine 0.05 per 1000 Gm. of body weight
- 3 rabbits treated with sulfadiazine 0.1 per 1000 Gm. of body weight
- 3 rabbits treated with sulfadiazine 0.2 per 1000 Gm. of body weight
- 3 rabbits treated with sulfadiazine 0.25 per 1000 Gm. of body weight

The weight of each animal was recorded twice weekly.

The urines were examined for blood and crystaluria during and after the period of drug administration. On the tenth day after cessation of drug administration the survivors were sacrificed. Autopsies were made on all rabbits which died during the experiment and on all sacrificed animals. Specimens of the tissues were fixed in 10 per cent formalin solution and stained by hematoxylin and eosin. In addition, deKossa's calcium stain was used in several cases according to Rake and co-workers,[1] Antopol, Lehr, and co-workers,[4] etc., who used this method for the determination of calcifying nephrosis in kidneys of animals which were damaged by sulfonamides.

Quantitative determinations of blood levels* were made in a separate series of rabbits receiving the chemical compounds under investigation in doses of 0.05 and 0.1 Gm. per kilogram twice a day. The purpose of this comparative blood level study was to obtain an idea of the speed of excretion of the various compounds during and after treatment. The bloods were taken immediately before the first drug administration (10 A.M.) on the day following the eighth dose, and twenty-four hours after discontinuance of drug administration.

Under the circumstances of this investigation sulfathiazole showed the lowest blood levels, and sulfadiazine, sulfapyrazine, and sulfamerazine approximately the same low blood levels, only somewhat higher than sulfathiazole.

RESULTS

Survivors.—At the end of the experiment, 30 of 48 rabbits survived as follows:

8 survivors of 12 rabbits after treatment with sulfathiazole.
7 survivors of 12 rabbits after treatment with sulfadiazine.
7 survivors of 12 rabbits after treatment with sulfapyrazine.
8 survivors of 12 rabbits after treatment with sulfamerazine.

*The blood levels were determined and various chemical compounds in this investigation supplied by the courtesy of Doctor G. W. Raiziss of the Dermatological Research Laboratories, Division of Abbott Laboratories, North Chicago, Ill., which is acknowledged with appreciation by the author.

All rabbits receiving doses of 0.05 or 0.1 Gm. per kilogram twice a day survived, remaining well, whatever compound was administered. At the dosage of 0.2 Gm. per kilogram twice a day none of the sulfadiazine rabbits survived, while one survivor was observed with sulfapyrazine, two with sulfathiazole, and two with sulfamerazine. The one of the two survivors given sulfamerazine showed extreme loss of weight, anorexia, weakness, and amotility at the end of the experiment. When doses of 0.25 per 1000 Gm. were given twice a day, none of the rabbits survived, except one of the sulfadiazine series. The deaths occurred from the fourth to the ninth day of drug administration except for one rabbit receiving sulfadiazine in dose of 0.2 per 1000 Gm., which died only four days after cessation of drug intake. The earliest deaths (on the fourth day of treatment) were observed with sulfadiazine (2 rabbits) and sulfamerazine (1 rabbit).

All rabbits dying during or after treatment showed rapid decrease of body weight, which became most evident when impairment of the urinary output indicated that severe kidney damage had been produced. Urinary block, with anuria during the last 36 hours before death, was observed with two rabbits of the sulfathiazole group and two of the sulfamerazine group, each animal receiving 0.25 per 1000 Gm. of drug twice a day (10 to 14 doses). The two animals of the sulfathiazole group showed amotility of the hind legs, tremor, and convulsions during the last 24 hours before death. Microscopic examination of specimens of the brains and spinal cords did not reveal pathologic changes. Hematuria was observed only microscopically in several rabbits of the various compound groups when the lethal dosage of 0.25 per 1000 Gm. twice a day had been used.

Crystaluria.—During drug administration crystaluria was observed with all four compounds when given for at least five to six days in doses of more than 0.05 per 1000 Gm. In the centrifugates of urine specimens collected over 18 to 20 hours significant crystal forms were seen which were decidedly different from all other crystals existing in the urines of normal rabbits. The number of these crystals apparently increased with higher doses of drug. After administration of sulfathiazole and sulfadiazine, we observed the characteristic crystals as described by Lehr and Antopol,[11] Prien and Frondel,[12] etc. After administration of sulfapyrazine, we found dark or muddy yellowish rhomboid-formed platelets, some of which showed indented outlines and globular forms. After sulfamerazine treatment, we observed crystals similar to the "shocks of wheat with eccentric binding" described by Lehr and Antopol[10] as crystals of acetyl-sulfadiazine.

Appearance of Precipitate in Tissue.—The precipitated particles found in the tubules consisted of amorphous or crystalloid material. Using high-power magnification (oil immersion) we never observed obvious crystalline forms except in sections from one rabbit dying after eight doses of 0.25 per 1000 Gm. of sulfathiazole. In this case we saw thin, glass-clear hexagonal platelets among amorphous masses of precipitate, the shape of which resembled the form of acetyl-sulfathiazole crystals photographed by Lehr and Antopol.[11]

AUTOPSIES

The pathologic changes of the organs observed at autopsy were largely confined to the kidneys, spleen, and liver, while the other organs generally

did not reveal significant pathologic changes. However, in one rabbit of the sulfamerazine group which died on the fifth day after eight doses of 0.25 Gm. of sulfamerazine per kilogram, extensive intestinal hemorrhages were found in the wall of the cecum. In this study concerning principally the nephrotoxic properties of the drugs tested, we are reporting in detail the findings only in both kidneys, liver, and spleen.

Kidneys.—In nearly all rabbits which survived the ten-day period of drug administration the appearance of the kidneys remained normal, when the dosage was not more than 0.5 or 0.1 Gm. per kilo twice a day. At that dosage only two survivors of the sulfathiazole series and one of the sulfamerazine series exhibited slight enlargement of one or both kidneys. In the rabbits which died after doses of 0.2 or 0.25 Gm. per kilo of the various compounds, the kidneys showed enlargement, edema, and hyperemia, the size varying from slight swelling to considerable enlargement up to twice the normal size. The color of these kidneys was either bluish-red or yellow-green, occasionally very pale, the consistency soft. Upon opening the kidneys, we found the medullar region congested, sometimes hemorrhagic; the cortical region usually pale, sometimes included in the dark red of the medullar region. Extreme enlargement was always associated with dilatation of the renal pelves, and occasionally of the proximal part of one or both ureters. Pus was found in the dilated renal pelves of two rabbits receiving doses of 0.2 Gm. of sulfadiazine per kilo, the one dying on the fifth day of administration, the other four days after discontinuance of the drug. In both animals hemorrhages were seen macroscopically in the medullar and cortical region; small particles of concrements were adherent to the walls of the renal pelves. Gross concrements (uroliths) up to the size of a cherry stone were found in the dilated renal pelves of five of the six rabbits receiving sulfamerazine in dose of 0.2 or 0.25 Gm. per kilo, four of which died during treatment. In some of these cases the concrements were large enough to obstruct the renal pelves; small particles obstructed the mouth of one ureter also. In one rabbit, given 0.25 per 1000 Gm. doses of sulfamerazine, small blood clots were found in the renal pelvis. Also one of the two survivors given doses of 0.2 Gm. of sulfamerazine per kilo, when sacrificed, showed obstructing uroliths, while the other survivor did not exhibit extrarenal concretion. In each of the five sulfamerazine rabbits which had stones in the renal pelves, gross particles of concretion were found embedded in the renal parenchyma also, with hemorrhages and tissue destruction around the foreign substance. This was also observed, although to a smaller extent, in the kidney of one rabbit dying after doses of 0.2 Gm. of sulfadiazine per kilo twice a day. Obviously in these cases, as Maisel and co-workers[3] described in dogs receiving sulfadiazine, parts of intratubular concretion had broken through the necrotic tubular walls so as to penetrate the surrounding organ tissue. Fig. 1 demonstrates the significant appearance of one kidney of a rabbit which received sulfamerazine and died on the seventh day (after 12 doses of 0.25 Gm. per kilo). Fig. 2 shows the form and size of the uroliths. The chemical analysis of crystals found in the kidneys of this rabbit and also the concretions found in the ureter disclosed the presence of the free and conjugated sulfamerazine. The conjugated material was several times larger than the free sulfamerazine.

At the same dosage of sulfapyrazine and sulfathiazole we found no gross concrements (uroliths) in the renal pelves or ureters.

In several survivors given the lower doses of 0.05 or 0.1 Gm. of drug per kilo, permanent dilatation of the renal pelves and the proximal part of the ureters, but with no concrements present, was observed at the end of the experiment. This occurred in survivors of both the sulfathiazole and the sulfadiazine group, but in none of the rabbits surviving after sulfapyrazine administration in equal doses.

Fig. 1.

Fig. 2.

The *bladders* of all animals dying during treatment were examined. Only small amounts of urine were present or else the bladders were empty. Only in one of the sulfamerazine rabbits given 0.25 Gm. per kilo twice a day, some particles of concrements were found in the bladder; the urine present contained red blood cells.

Frequently marked differences were observed in the extent of injury of the two kidneys. While the one kidney at times showed severe change, the other showed only slight or moderate change.

Spleen and *liver* did not reveal gross changes from the normal when doses of 0.05 or 0.1 Gm. per kilo were used. After higher doses, namely 0.2 or 0.25 Gm. per kilo, changes in size, color, and consistency were observed frequently. When involved, the spleens were enlarged and hyperemic. Extremely small spleens were found in two rabbits, the one surviving sulfadiazine doses of 0.25 Gm., the other sulfamerazine doses of 0.2 Gm. per kilo. The livers, when involved, were enlarged, of a yellowish color, and of soft and fragile consistency.

MICROSCOPIC

Kidneys.—The pathologic tissue changes produced in the kidneys of laboratory animals by prolonged sulfonamide administration present a characteristic appearance. Detailed descriptions have been published in the literature.[1, 3-6] Corresponding to the varying dosage, and in varying degree with the four compounds, we were able to observe the gradual development of the renal injury. The first evidence of injury was revealed in the cells of the lining epithelium of the convoluted tubules, the walls of which were exposed to the pressure of insoluble precipitate irritating and finally damaging the epithelial cells. In the afflicted tubules these cells appeared cloudy, swollen, granular or with small irregular vacuoles, the outline indistinct, the nuclei partly pale, partly fragmented; eventually the absence of the nuclei was encountered in many areas. Because of the accumulation of precipitate, the tubules were dilated, occasionally extremely dilated. Cellular and albuminous casts were seen in many tubules which had completely lost their epithelial lining; only cell debris and precipitate particles were found free in the tubules. Chiefly the convoluted tubules were injured; to a small extent also, Henle's tubules were at times involved, while the collecting tubules appeared either undamaged or only slightly affected, showing some cloudy swollen cells with indistinct outlines. Obvious lesions of the tubules usually were associated with dilatation of the glomerular spaces which sometimes contained a few precipitate particles. In rabbits which died after doses of 0.25 per 1000 Gm. of drug, we found extreme capsular dilatation, some glomeruli filling only a fifth or less of the space. Hemorrhages were found in the renal tissue of those animals in particular in cases where insoluble concretion had broken through the destroyed tubular walls, producing focal parenchymatous degeneration. In kidneys of rabbits which died after the first five days of drug intake, leucocytic infiltration was found to a varying extent around the damaged tubules.

In sections of rabbits which died during treatment extremely dilated tubules were found with no precipitate present. Obviously the crystalline masses had been partly removed by excretion before death; on the other hand, some precipitate may have been washed out during fixation of the specimens. We refer to the detailed descriptions by Rake et al.[1] in mice, Sobin et al.[9] in rats.

Our results summarized in Tables I and II refer to the extent, site, and degree of the pathologic changes which we observed in our sections. In every case both kidneys were examined microscopically. The symbols used indicate the degree of damage in the tissue observed with the various dosages of drug.

From the tables it will be observed that severe changes were found in all rabbits dying after the usually lethal dosage of 0.25 Gm. per kilo twice a day,

TABLE I

PATHOLOGIC TISSUE CHANGES OF THE KIDNEYS AFTER PROLONGED ADMINISTRATION OF SULFAPYRAZINE AND SULFAMETHYLDIAZINE (SULFAMERAZINE) AS COMPARED WITH SULFATHIAZOLE AND SULFADIAZINE

RABBIT NO.	DOSE PER KILO (GM.) TWICE PER DAY ORALLY	SULFATHIAZOLE				SULFADIAZINE			
		SURVIVORS (S)	DEATHS AFTER DAYS OF TREATMENT	DEGREE OF PATHOLOGIC CHANGES OF KIDNEY TISSUE	ADDITIONAL REMARKS UROLITHS (U)	SURVIVORS (S)	DEATHS AFTER DAYS OF TREATMENT	DEGREE OF PATHOLOGIC CHANGES OF KIDNEY TISSUE	ADDITIONAL REMARKS UROLITHS (U)
1	0.05	S	--	0	--	S	--	0	--
2	0.05	S	--	+	--	S	--	+	--
3	0.05	S	--	0	--	S	--	+	--
4	0.10	S	--	0	--	S	--	+	--
5	0.10	S	--	0	--	S	--	0	--
6	0.10	S	--	0	--	S	--	0	--
7	0.20	S	--	+	--	--	10	+++	Pyelitis and U
8	0.20	S	--	0	--	--	3	++	--
9	0.20	--	5	++	--	--	4	+++	Pyelitis and U
10	0.25	--	4	++	--	--	3	+++	--
11	0.25	--	5	+++	--	--	5	+++	--
12	0.25	--	6	++	--	S	--	++	--

0 indicates: no gross or microscopical changes from the normal.

+ indicates: dilatation of collecting tubules limited to small areas, with scarce foci of epithelial necrosis, occasionally with small amounts of precipitate free in the tubules.

++ considerable dilatation of convoluted and collecting tubules with focal necrosis of the lining epithelium, precipitate accumulated in the tubules, and cellular casts, some hemorrhages; in some areas dilatation of glomerular spaces.

+++ indicates: extreme dilatation of the glomerular spaces and of the convoluted and collecting tubules showing extensive epithelial necrosis, immense amounts of precipitate or gross concrements (uroliths), hemorrhages, occasional leucocytic infiltration around the afflicted tubules, and presence of numerous albuminous and cellular casts.

TABLE II

RABBIT NO.	DOSE PER KILO (GM.) TWICE PER DAY ORALLY	SULFAPYRAZINE				SULFAMERAZINE			
		SURVIVORS (S)	DEATHS AFTER DAYS OF TREATMENT	DEGREE OF PATHOLOGIC CHANGES OF KIDNEY TISSUE	ADDITIONAL REMARKS UROLITHS (U)	SURVIVORS (S)	DEATHS AFTER DAYS OF TREATMENT	DEGREE OF PATHOLOGIC CHANGES OF KIDNEY TISSUE	ADDITIONAL REMARKS UROLITHS (U)
1	0.05	S	--	0	--	S	--	0	--
2	0.05	S	--	0	--	S	--	0	--
3	0.05	S	--	0	--	S	--	+	--
4	0.10	S	--	0	--	S	--	+	--
5	0.10	S	--	+	--	S	--	++	--
6	0.10	S	--	+	--	S	--	+	--
7	0.20	--	7	++	--	S	--	+	--
8	0.20	S	--	+	--	S	--	+++	U
9	0.20	--	8	++	--	--	7	+++	U
10	0.25	--	4	+++	--	--	4	+++	U
11	0.25	--	5	++	--	--	3	+++	U
12	0.25	--	6	+++	--	--	6	+++	U

sulfamerazine producing the highest injury. Marked differences concerning the various compounds became evident when the sublethal dosage of 0.2 Gm. per kilo had been used. At this dosage the sulfathiazole group exhibited obviously less tissue damage than did the other compound groups, and the sulfapyrazine group less than did sulfamerazine and sulfadiazine. After use of the lower doses, i.e., 0.05 or 0.1 Gm. per kilo twice a day, no change from the normal was observed with any compound, except for an occasional focus of epithelial irritation or degeneration in a few convoluted tubules. Finally, it has to be noted that also in one of both survivors of the sulfathiazole group receiving 0.2 Gm. per kilo twice a day, no pathologic change was revealed at the end of the experiment; the other survivor of this group and one survivor of both the corresponding sulfapyrazine and sulfamerazine groups exhibited only slight pathologic change.

Calcium deposits were revealed by deKossa's stain in the kidneys of all rabbits receiving drug in doses of 0.2 or 0.25 Gm. per kilo for at least seven days. At this dosage we observed the beginning of calcium deposit upon necrotic parts of the tubular epithelium or around cell debris and precipitate particles free in the tubules. However, after administration of sulfathiazole, sulfapyrazine, and sulfadiazine, only slight calcium deposits were found in the cortical region. More advanced calcification became evident only in specimens of two rabbits receiving doses of 0.2 Gm. of sulfamerazine per kilo, the one dying after seven days of drug intake, the other when sacrificed.

Spleen and Liver.—When doses of 0.05 or 0.1 Gm. per kilo twice a day were used, no pathologic changes were observed with any of our compounds. At the dosages of 0.2 or 0.25 Gm. per kilo damage was observed with all compounds. When involved, the *spleens* showed marked changes limited to the Malpighian corpuscles with considerable to extreme reduction of the lymphocytes of the mantle zone and hyperplasia or, in some areas, complete disappearance of the germinal centers. Complete reduction of the lymphocytes of the mantle and marginal zones, with nothing remaining but collapsed stroma and a few pyknotic cells, was seen in the spleens of rabbits which died at the dosage of 0.25 per 1000 Gm. of the four drugs, and in two rabbits given 0.2 per 1000 Gm. of drug twice a day, the one belonging to the sulfapyrazine, the other to the sulfamerazine group.

The *livers,* when involved, showed vacuolization or complete degeneration of the peripheral liver cells of some lobules. The greatest development of liver cell damage was found in cases where obstruction of the renal passages had impaired the excretory function. Severe changes in spleen and liver obviously corresponded to the degree of kidney injury.

DISCUSSION

The nephrotoxic properties of sulfonamide derivatives are due principally to a mechanical effect of the insoluble drug precipitate which irritates and finally destroys the lining epithelium of the tubules by pressure necrosis, and simultaneously obstructs the renal passages.[6, 7, 9*] The increase in precipitate masses which cannot be excreted as rapidly as formed in the convoluted tubules

*This fact does not exclude the possibility that after chronic administration of generally toxic doses the mechanical injury may be associated sometimes with a direct toxic effect on the parenchyma of the kidneys.

suppresses the urinary output and, as a result, may cause urinary block. This fatal symptom is most common when gross uroliths have been formed in the renal pelves barring the urinary passage. In this respect the varying solubility of the drugs and their acetylated products is of great importance. For instance, poor solubility combined with slow excretion is a factor which can favor the formation of gross concrements (uroliths).

For this reason, one can expect with justification that rabbits, which have a great ability to acetylate sulfonamide derivatives, will be more apt to have gross concrements in the kidneys after prolonged administration of sulfonamides, which form extremely insoluble acetylated products. Insofar as the relationship between precipitation and solubility is concerned, fewer gross concrements (uroliths) are to be expected in rabbits after prolonged administration of sulfonamide derivatives, which have acetylated products that are more soluble.

As reported by Hamburger, Rueggsegger, et al.[15] on sulfapyrazine and sulfadiazine, and by Welch, Mattis, et al.[21] on sulfadiazine and sulfamethyldiazine (sulfamerazine), the acetylated derivatives of these compounds proved to be more soluble than the unacetylated drug; and, as to sulfamerazine, both sulfamerazine and its acetylated products proved to be somewhat more soluble than the respective sulfadiazine (in urine at a pH of 7 or less); furthermore, the degree of acetylation was the same with sulfamerazine as it was with sulfadiazine.[21-23] From their experience with animals and human beings and their results in comparative blood level studies, Welch and his group concluded that sulfamerazine is more rapidly and more completely absorbed from the gastrointestinal tract and yet more slowly eliminated by the kidneys than is sulfadiazine at equal dosage. Despite their observation that the blood reaching the kidney was very much lower in its sulfadiazine content than it was in sulfamerazine content, these authors observed that more sulfadiazine than sulfamerazine was excreted in the urine during the first twenty-four hours. These investigators used various species of laboratory animals in their experiments, i.e., mice, rats, chickens, dogs, and monkeys. In our comparative study on kidney damage following prolonged drug administration, we used rabbits only. Under the conditions of our experiment we observed that, insofar as renal precipitation is related to solubility, sulfamerazine apparently is able to produce more kidney damage in rabbits than do sulfadiazine, sulfapyrazine, and sulfathiazole at equal dosage, because of the great ability of sulfamethyldiazine to form intrarenal and extrarenal concrements in rabbit kidneys. This was observed with five of six rabbits given 0.2 or 0.25 Gm. of sulfamerazine per kilo twice a day, in comparison with two of six rabbits which were given sulfadiazine in equal doses. The two rabbits of the sulfadiazine series showed only a few small particles of concretion in the renal pelves. Neither with sulfapyrazine nor with sulfathiazole did we observe the formation of uroliths deposited in the renal pelves of rabbits.

In evaluating the extent and degree of the kidney injury observed under the conditions of our experiment we had to consider not only the site and extent of organ changes found in rabbits which died during drug administration, but also the permanent tissue change observed in the sacrificed survivors.

When we sacrificed the rabbits on the tenth day after receiving the last dose of 0.05 or 0.1 Gm. per kilogram of drug, we found no remaining path-

ologic change in the kidneys except for an occasional focus of tubular epithelial necrosis. When doses of 0.2 Gm. per kilogram were used, sulfathiazole apparently caused less tissue changes in the kidneys of the surviving animals than did the other compounds. The one survivor receiving doses of 0.2 Gm. of sulfapyrazine per kilo and the one of the two survivors treated with equal doses of sulfamerazine showed lasting kidney damage of only a small degree, while the other sulfamerazine survivor exhibited severe kidney damage, as we described. At equal dosage, there were no survivors in the corresponding sulfadiazine group. Surprisingly, one rabbit receiving the highest dosage of sulfadiazine, namely 0.25 Gm. per kilo, survived, showing only a moderate extent of tissue change; this was the only one of all rabbits used for our experiment which survived the ten-day administration of 0.25 Gm. of drug per kilogram twice a day.

The deposit of *calcium,* as revealed by deKossa's calcium stain, cannot be considered as specific for sulfonamide injury, but only as a "general response of the kidneys to injury" (Sobin and co-workers[9]). The demonstration of calcification, however, has been generally acknowledged as an index of permanent tissue damage insofar as calcium deposit usually corresponds to the extent and site of necrotic cell substance or other foreign material existing in the organ tissue.

The presence of sulfonamide *crystals,* which we found in the urines of our rabbits during drug administration, did not give any indication of injury as long as the output of urine appeared normal and the urine did not show blood content macroscopically or microscopically.

The pathologic changes observed in *spleen* and *liver* generally corresponded to the degree of impairment of the renal function. The accumulation of toxic amounts of drug in the blood and liver may be responsible for the pathologic tissue changes observed particularly in the livers.

For this comparative study we considered only the nephrotoxic effect after prolonged drug administration as the basis for evaluation of the compounds. Since the number of animals used for our investigation was limited to three rabbits for each dosage, we do not feel justified in basing conclusions concerning the toxicity on the number of survivors.

CONCLUSIONS

Under the conditions of our experiment, sulfapyrazine and sulfamethyldiazine (sulfamerazine), when given in doses of not more than 0.05 or 0.1 Gm. per kilogram twice a day for ten days in succession (20 doses), did not produce marked tissue damage in the kidneys of rabbits, except for an occasional focus of tubular epithelial necrosis in a few areas. The same results were obtained with sulfathiazole and sulfadiazine. All rabbits in our experiments receiving doses of 0.05 or 0.1 Gm. per kilogram survived.

When doses of 0.2 or 0.25 Gm. per kilogram were given, all compounds showed nephrotoxic effect in varying degrees.

Sulfathiazole in dosage of 0.2 Gm. per kilogram produced a lesser degree and lesser extent of kidney damage than did the other compounds. Sulfapyrazine produced less kidney damage than did sulfadiazine or sulfamerazine.

Sulfamerazine, in doses of 0.2 or 0.25 Gm. per kilogram, showed great ability to cause the formation of gross intrarenal and extrarenal concrements (uroliths) as observed in five of six rabbits. This was not observed with sulfapyrazine and sulfathiazole at equal dosage and only in two of six rabbits with sulfadiazine, where the extrarenal concrements in the renal pelves were small and scarce.

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THE DIAGNOSIS AND TREATMENT OF EPIDEMIC CEREBROSPINAL MENINGITIS

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WHAT little justification there may be for presenting another paper on meningococcic infections arises first from the conviction that the necessity for an early diagnosis can never be overemphasized, and second from a feeling that the knowledge acquired from the use of the newer forms of chemotherapy should be disseminated.

ANALYSIS OF OUR CASES

From Dec. 1, 1942, to Aug. 20, 1943, forty-one patients with a meningococcus infection entered the Station Hospital at Fort Custer, Michigan. These fell into two groups, namely those with meningitis and those with a meningococcemia without meningitis.

Group I.—In this group with meningitis there were 35 patients, all of whom had leucocytes in the spinal fluid. In 33 of these the organism was recovered from the spinal fluid and in 8 of the 33, it was recovered from the blood as well. In the remaining 2 patients, the spinal fluid failed to reveal the organisms, but the blood cultures were positive.

Of the total number, 12 were classified as mild, 10 as moderately severe, 10 as severe, and 3 as fulminating. Classified as fulminating were patients who displayed signs of hyperirritability, had headache and vomiting, and who passed into coma few hours after the onset of these symptoms. One such patient, awaiting discharge from the Army because of bilateral pulmonary atrophy due to old empyemata, died forty-eight hours after onset with hemorrhages into the adrenals. Another patient whose headache occurred nine hours previous to admission remained in coma until death, sixty-six hours after entering the hospital. Autopsy revealed multiple petechiae throughout the brain with just a vestige of cortical exudate. The third patient lapsed into coma five hours after the onset of headache, but recovered.

Group II.—In this group without meningitis there were 6 patients. The spinal fluids of all were completely negative, but their blood cultures were positive. Four of these had headache, weakness, some stiffness of the neck, and a macular eruption. One of these 4 recovered without treatment while awaiting report of his blood culture, which did not show a growth until the fourth day.

Two of the 6 had a septic type of fever and had a fleeting recurring macular eruption without any other signs indicative of meningococcemia or meningitis. In these 2 the blood culture proved positive on the fourth and sixth days respectively. In retrospect, we believe that in a number of patients running such a course the diagnosis was overlooked either because the spinal fluid and

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blood culture were negative or because of failure to bear in mind the possibility that such a symptom complex could spell meningococcic infection.

The meningococci recovered in both groups were identified as Type I. The mortality rate for both groups was 5 per cent. If one excludes Group II which embraced those patients having only a meningococcemia, the mortality rate was 5.7 per cent.

In the majority of the patients in both groups the signs and symptoms were characteristic enough to warrant a diagnosis on clinical grounds while in the others they were only suggestive. For example, in 6 patients the neck rigidity was completely absent and in 7 only suggestive. A skin rash was present in 18 patients. In 6 patients the Kernig and Brudzinski signs were positive and in 14 only suggestive.

In 21 of the 41 patients the signs and symptoms of the prodromal stage differed little from those observed in our epidemics of either respiratory tract infection or of measles. Scarlet fever, streptococcic sore throat, reaction from typhoid fever, and lymphocytic choriomeningitis, of which there were 5 cases, sometimes had initial symptoms difficult to distinguish from the prodromata of meningococcus infection.

Thus can be seen the preplexity attending the problem of making an early diagnosis of meningococcic infection. This is better emphasized by stating that during the above-mentioned period 539 patients, presenting signs or symptoms of cerebrospinal fever, were admitted to the ward set aside for "suspects." In 72 of these "suspects," the findings were suggestive enough to warrant a spinal puncture.

CRITERIA FOR DIAGNOSIS

In searching for criteria which enabled us to arrive at a presumptive diagnosis before frank meningitis became evident, we selected the following signs as being the earliest and most outstanding.

Headache was among the earliest if not the earliest symptom present. Generally located in the frontal and parietal regions, it was described as severe, persistent, and pounding, gradually becoming intolerable. Almost always, it was accompanied by photophobia. Weakness and instability following upon the headache were constantly present and often associated with generalized aching and muscle soreness.

Vomiting, rarely absent, likewise was an early sign having occurred previous to admission in 22 of our patients. It was rarely projectile and often was preceded by a short period of epigastric uneasiness.

Stiffness of the neck, in contradistinction to the pronounced neck rigidity of severe or advanced meningitis, was present to some degree in 31 of our patients. It was never complete nor was it constant. Frequently it was apparent only in the last third of flexion.

A skin eruption was present in 18 patients. Neither striking nor extensive, it consisted of rose-colored macules, maculopapules, and some petechiae. It was distributed over the trunk, forearms, or ankles. In only 2 patients was it marked enough to be characterized as that of "spotted fever."

The Kernig and Brudzinski signs as well as the prodromata of nasopharyngitis were left out of consideration as early signs.

In no patient presenting the above symptom complex did we fail to find evidences of meningococcic infection either in the spinal fluid or in the blood stream. The headache combined with any other two of the findings always was considered an indication for a spinal puncture and a blood culture.

TREATMENT

Successful treatment depends upon two factors. First, immediate recognition of the infection, and, second, the promptness with which treatment is begun. Severity of the infection may be considered of lesser importance, it having been our observation that even the severe cases, and those recognized late, respond to prompt and vigorous therapy. The fulminating case, too, as seen in our series, occasionally responds. Therapy, primarily and always, is the treatment of the patient, not the disease. Recognizing this dictum we followed no routine, but adopted certain broad principles as guides.

When a patient admitted was suspected of having a meningococcus infection, a spinal puncture was performed, blood was drawn for culture, and a nasopharyngeal culture was made. Upon completion of these procedures, treatment was started without waiting reports of the laboratory, it being our conviction that the disease should be considered in the light of an emergency.

The drug treatment was confined to the use of sodium sulfadiazine, and sulfadiazine. With respect to the dosage and frequency of administration, this was judged almost solely by the clinical response. The drug was continued three to five days after maximum clinical improvement. The average duration of therapy was ten days.

In the milder cases sulfadiazine was given orally unless nausea or vomiting was present. The initial dose was 2 Gm. followed by 1 Gm. every four hours thereafter for from two to five days. After that it was discontinued during the night.

In the severer cases the initial treatment was always given intravenously, the dose being 5 Gm. of sodium sulfadiazine in 200 c.c. of distilled water. This was repeated in four hours if the vomiting or toxicity was extreme. In very severe cases even a third dose intravenously had to be given. During the first twenty-four hours after discontinuance of intravenous treatment, the sulfadiazine was given orally in 1 or 2 Gm. doses every two, three, or four hours. Subsequent treatment was determined by clinical response, side reactions of the drug, and blood level. If clinical improvement was satisfactory, sufficient sulfadiazine was given to maintain a blood level of about 4 or 5 mg. per 100 c.c. Where improvement was felt to be too slow, an effort was made to bring the level up to 12 to 14 mg. per 100 c.c.

Intravenous or subcutaneous salt or 5 per cent dextrose solution was given when dehydration as evidenced by dryness of the tongue and a urinary output of less than 1000 c.c. per twenty-four hours was present. Efforts were made to maintain the urinary output as near 2000 c.c. as possible.

Sedation with analgesics was used freely. The barbiturates, then paraldehyde, and if necessary morphine in the order of choice were the sedatives used.

CONTROL MEASURES

Progress in therapy was judged by the clinical response; namely, an abatement of the headache, a fall in temperature, freedom from nausea and vomiting, and lessening of the neck rigidity. The blood sulfadiazine level was used as an index of the intensity of therapy. No effort was made to maintain a fixed level as it was found that neither the exact height nor constancy of the level run parallel with clinical improvement or bacteriostatic response.

Patients severely ill often showed as marked a clinical improvement and bacteriostatic response when the sulfadiazine level was maintained at 5 mg. per 100 c.c. as at 15 mg. per 100 c.c. This is illustrated by Table II, in which, with a blood sulfadiazine level of 7.05 during the second twenty-four-hour period of the disease, the spinal fluid smears and cultures were negative and the clinical signs disappeared or regressed. This is further emphasized by Table I which represents the sulfadiazine level at the end of the first twenty-four-hour period in 4 patients who ran a parallel course, and who showed about the same clinical improvement and bacteriostatic response.

TABLE I

VARIATIONS IN THE BLOOD SULFADIAZINE LEVELS IN PATIENTS SHOWING ABOUT THE SAME CLINICAL IMPROVEMENT AND BACTERIOSTATIC RESPONSE

NAME	GRAMS SULFADIAZINE FIRST 24 HOURS	SULFADIAZINE BLOOD LEVEL AFTER FIRST 24 HOURS
R. S.	11.0	8.4 mg. per 100 c.c.
O. C.	11.0	26.9 mg. per 100 c.c.
J. M.	11.0	10.6 mg. per 100 c.c.
C. B.	11.0	2.5 mg. per 100 c.c.

Sulfadiazine blood level determinations were done every other day, or oftener, depending upon the severity of the cases.

TABLE II

FINDINGS IN ONE PATIENT WHICH MAY BE TAKEN AS REPRESENTATIVE OF THE GROUP

		ADMISSION	FIRST 24-HR. PERIOD	SECOND 24-HR. PERIOD	THIRD 24-HR. PERIOD
Spinal Fluid Findings	Appearance	Cloudy	Turbid	Opalescent	Clear
	W. B. C.	5250	4710	1010	452
	Neutrophiles per cent	67	91	94	15
	Lymphocytes per cent	33	9	6	85
	Smear	+	+	0	0
	Culture	+	0	0	0
	Sugar in mg. per 100 c.c.	Trace	33.0	37.0	50
	Sulfadiazine level in mg. per 100 c.c.	0	4.4	6.0	12.7
Clinical Signs	Headache	+++	±	0	0
	Neck stiffness	+	+	±	0
	Rash	+	±	0	0
	Kernig's sign	0	0	0	0
Sulfadiazine	Total amount of sulfadiazine given in each 24-hour period	0	7 Gm.	6 Gm.	6 Gm.
	Grand total in the three 24-hour periods				19 Gm.
	Sulfadiazine level in mg. per 100 c.c.		8.8	7.50	15.0

Spinal punctures were done every twenty-four hours in some of our patients as a matter of interest to determine the effects of therapy. The record of the findings in one of our patients, as shown in Table II, is representative of the group.

The fluid intake and output were measured each day. Urinalyses were done every other day with an eye to the presence of albumin, red blood cells, and sulfadiazine crystals. A white blood count and hemoglobin determination were made every other day, as an index of a toxic effect on the bone marrow.

Any tendency to neutropenia or impaired kidney function was met by a reduction in the dosage of the drug and by venoclysis.

DRUG COMPLICATIONS

Two of our patients had hematuria and some nitrogen retention. One of these patients also had an oliguria. Another patient developed drug fever. In one of our fulminating cases, where the blood sulfadiazine level was pushed to 29 mg. per 100 c.c., the urinary output dropped to 450 c.c. during the twenty-four-hour period preceding his death, during which period he had received 2500 c.c. of isotonic salt solution parenterally. On the day preceding, his urinary output was 1450 c.c. with a total fluid intake of 4000 c.c. There were some amorphous deposits in the bladder, ureters, and kidneys, and a blocking of some of the tubules.

SUMMARY AND CONCLUSIONS

Of the 41 patients observed with a meningococcus infection, 33 had pus cells and organisms in the spinal fluid; 2 with a positive blood culture had pus cells, but no organisms in the spinal fluid; and 6 had a completely negative spinal fluid, but a positive blood culture.

The presence of headache, weakness and instability, vomiting, stiffness of the neck and a skin eruption were the five earliest signs present and were considered to be evidence presumptive of the presence of meningococcus infection. The presence of headache with any two of the other findings was considered an indication for spinal puncture.

Treatment was started immediately after spinal puncture without awaiting laboratory reports. Progress was determined by clinical response. In the management of these cases limited dependence was placed on the blood sulfadiazine level.

The mortality rate for the entire series was 5 per cent.

THE ASSOCIATION OF ACUTE INTERSTITIAL PANCREATITIS WITH ACUTE PNEUMOCOCCIC MURAL ENDOCARDITIS*

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THE coexistence of two clinical entities is not uncommon, but where the clinical and pathologic entities are of unusual occurrence and very difficult of diagnosis, such a case, we feel, merits recording. It is with this view in mind that we wish to report the following case:

M. B., 47, housewife, was admitted to Medical Service 2 at the Mount Sinai Hospital on the fifth of July, 1938, with the following history. On the 18th of June she was seized with severe abdominal pain centering in the epigastrium, radiating to both hypochondrial regions, and associated with nausea, vomiting, and fever. She was sent to the Philadelphia General Hospital where she was studied for a period of ten days. Studies there revealed a leucopenia on three blood examinations, sterile blood culture, and normal blood chemistry findings. Two roentgenograms suggested a pneumonic lesion on the right side. X-ray of the gastrointestinal tract was negative. No definite diagnosis was established. The symptoms persisted. She insisted on being transferred to the Mount Sinai Hospital where she had been treated for a pleuropneumonia of the right lung five years previously.

On admission, she appeared very ill, pale, restless, somewhat dyspneic, and markedly dehydrated. There was evidence of an acute upper respiratory infection, harsh and prolonged expirations were heard, and impaired resonance with showers of crackling râles were detected over the right lower lobe. The heart was rapid but presented no other abnormalities. The abdomen was distended, tender, and tympanitic. Peristalsis was audible. Vomiting was one of the most annoying of her symptoms. Blood pressures were 120/85; temperature, 101° F.; pulse, 110; respirations, 36. Because of the predominance of the abdominal symptoms and signs, a provisional diagnosis of an "acute abdomen," most likely pancreatitis complicated by pneumonitis, was made. She was given fluids and electrolytes by hypodermoclysis and studies were undertaken.

The hemogram revealed a hemoglobin of 60 per cent (Sahli), 8.7 Gm.; red blood cells, 3,500,000; white blood cells, 11,200, of which 84 per cent were polymorphonuclear neutrophiles and of which 47 per cent were young forms. Blood platelets numbered 240,000. Blood cultures were sterile. Blood urea nitrogen was 13 mg.; blood cholesterol, 182 mg.; blood sugar, 145 mg. per 100 c.c. of blood; urinary diastase present in dilution, 1:80; serum lipase reading was 0.85 c.c., icteric index, 8.4 units; urea clearance, 58 per cent. The urine showed a trace of protein. Serology was negative. The sputum contained no pneumococci. Staphylococci and nonhemolytic streptococci were present. X-ray of the chest was negative.

Within a few days the abdominal symptoms subsided and the patient felt much better. However, within three days, toxicity again became manifest; pyrexia assumed a high level, with pulse and respirations in proportion. Within ten days a palpable mass was felt in the left hypochondrium. This was considered to be an enlarged spleen, although the possibility of an enlarged kidney was also considered. This enlargement occurred after a blood transfusion. The blood picture remained the same. The possibilities of typhoid, submiliary tuberculosis, and brucellosis were considered. Agglutination tests of the blood, urine,

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and feces examinations suggested no specific diagnosis. Intravenous urography revealed a normally sized and placed kidney with a suggestion of dilatation of the calices in the right kidney. X-ray of the lungs revealed congestion but no consolidation. The hemograms on 7/19/38, a day before death, revealed a leucocytosis of 29,100 with 90 per cent polymorphonuclear neutrophiles, of which 82 per cent were segmented. The blood urea nitrogen at that time was 15 mg. per 100 c.c. of blood. In spite of transfusion and general care the toxicity continued. Her temperature reached 105° to 106° F. Signs of meningeal irritation appeared as manifested by nuchal rigidity. She died on 7/20/38 before a spinal puncture could be done, fifteen days after admission.

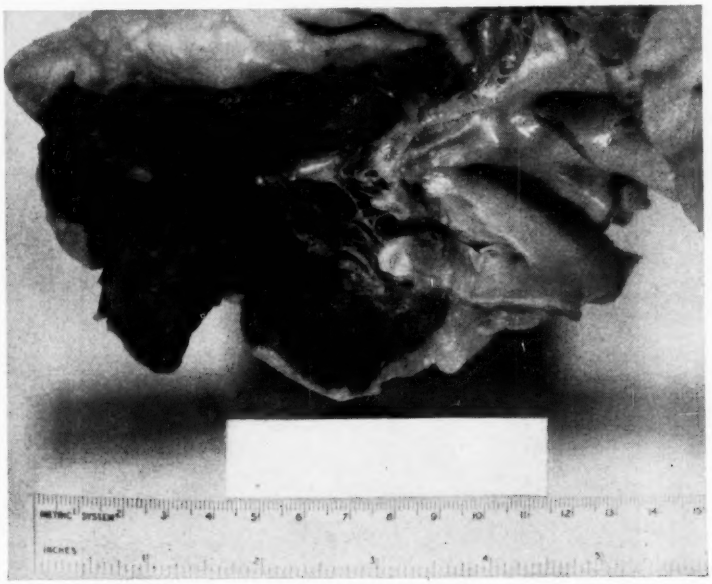

Fig. 1.—Heart showing mural thrombus. (Section taken through site of thrombus.)

Pathologically the underlying finding at autopsy was a pneumococcic Type III septicemia. Abscesses from which this organism was isolated were present in the lungs, heart, spleen, kidney, liver, and perirenal fat tissue. In addition to these findings, three totally unexpected changes were observed at autopsy, the gross pathologic findings in the spleen and heart and the microscopic findings in the pancreas. During life, as already stated, a mass was palpated in the left upper quadrant. This proved at autopsy to be a spleen roughly three times normal size, whose surface was covered with fibrinous exudate. The enlargement of the organ was largely due to the replacement of the midportion of the spleen by a large cavity filled with broken-down splenic tissue and blood. The lining of this pseudocyst was ragged and histologically consisted of broken-down splenic tissue. Several of the branches of the splenic artery were thrombosed. In the absence of a more satisfactory explanation, we believe that these splenic changes were caused either by the rupture of an intrasplenic vessel or by hemorrhagic liquefaction of an area of early infarction.

The most unexpected finding was the presence of a mural thrombus involving the left auricle, slightly beyond the line of attachment of the posterior mitral leaflet (Fig. 1). The vegetation covered about 1.5 cm. of the auricular wall. It was raised but flat and overlay an auricular wall honeycombed throughout almost its entire thickness by a suppurative process. The mitral valve leaflets themselves were moderately thickened but not deformed. Histologically there were no evidences of an active inflammatory process in the valve leaflets. The pathogenesis of this vegetative process becomes a matter for pathologic speculation. The heart muscle showed histologically an active acute diffuse and focal suppurative process. It is probable that the suppurative process in the region of the thrombus had destroyed surface myocardial cells and provided the setting for the thrombus.

The third unexpected finding was the presence of pancreatic changes. The pancreas shows diffuse edema (Fig. 2). In many regions there was also small focal infiltration, and in other areas more diffuse infiltration with polymorphonuclear cells and lymphocytes (Fig. 3). In some areas the infiltration was predominantly lymphocytes. In still other such regions fibrous tissue cells were present in increased numbers.

In addition, the lungs presented multiple abscesses, one large abscess in the left upper lobe and in some areas an unresolved pneumonic process. The kidneys showed widespread suppuration. The brain showed a diffuse suppurative meningitis, particularly marked in the basal regions. The cerebrospinal fluid was turbid and contained flocculi. A pneumococcus Type III was obtained on culture of the meningeal exudate.

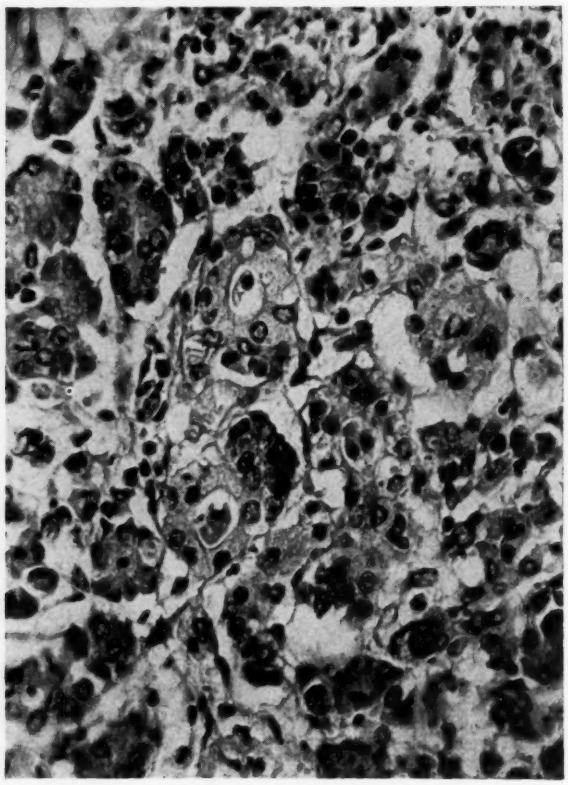

Fig. 2.—Pancreas—diffuse edema. ×400.

DISCUSSION

The incidence of acute pneumococcic endocarditis as reported in the literature varies from 0.06 to 2 per cent. Ruegsegger[1] reports an incidence of 2.9 per cent of endocarditis in her series of acute pneumococcic infections.

Pneumococcic endocarditis may complicate pneumonia at any stage of the illness or days or weeks after an apparently uneventful convalescence. Six of Ruegsegger's patients were afebrile from two to seven days when the complication manifested itself by sudden chills and fever. It differs from subacute bacterial endocarditis in the following ways:

1. It affects older age groups.
2. It may involve a perfectly normal endocardium.

3. Superficial embolism is very rare, but abscess embolism, usually purulent in character, is common.

4. Purulent meningitis is a frequent complication.

5. The course of the disease is rapid. Death takes place within four or five weeks.

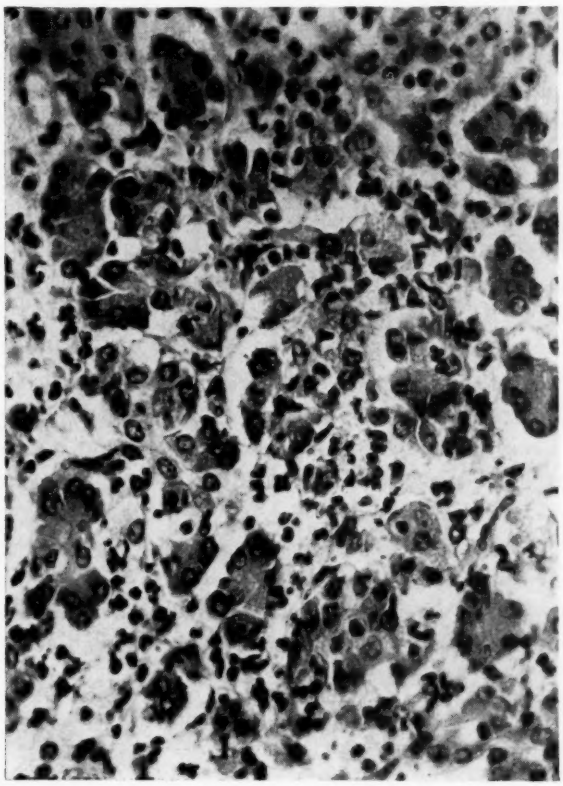

Fig. 3.—Pancreas—diffuse infiltration with polymorphonuclear white blood cells. ×400.

Mural endocarditis has been reported, but it is much less common than the valvular types. In Ruegsegger's series only three were noted and even in these the process extended from the valves onto the mural endocardium. Acute bacterial endocarditis is a part of an overwhelming general infection, so that the septicemia overshadows the endocardial manifestation. This is particularly so where the pathology is mural in type. Hence the diagnosis is usually made at post mortem.

Acute interstitial or acute edematous pancreatitis is a definite clinical entity. It runs a milder course than that of acute pancreatic necrosis and recoveries are the rule.

Elman's[2] description of the histologic picture of this disease is worth quoting. He writes: "The striking finding was the marked infiltration of acute (polymorphonuclear) inflammatory cells into the interstitial tissue between lobules as well as acini, often with evidence of edema as shown by the outpouring of fluid." In another publication[3] two years later he expresses the same

opinion. Cole's[4] description of this condition coincides with that of Elman. The pathologic findings justify our clinical diagnosis in this case. One may ask whether the pancreatic lesion is not part of the generalized infection. It seems to us that the nature of the pathologic process in the pancreas, i.e., the absence of any gross suppurative process, would probably speak against such a possibility.

If one is justified in speculation, and one is frequently compelled to speculate in clinical medicine, there is a strong possibility that the acute pancreatitis was the initial pathologic lesion. The suddenness of the onset with predominantly abdominal symptoms and the abdominal signs are typical of acute pancreatitis. While it is true that pneumonia may be ushered in with abdominal signs, the severity and persistence for almost two weeks would rather speak against such a possibility. It is logical to consider that the lowered resistance caused by starvation and dehydration due to the pancreatitis prepared the soil for the invasion of the Type III pneumococcus with its subsequent multiple suppurative lesions. One of Elman's autopsy reports is from a patient who died from another cause some weeks after the attack of acute pancreatitis.

This case possesses the criteria differentiating acute bacterial from subacute bacterial endocarditis. She was 47 years old, there was no evidence of any previous heart disease, and the emboli were limited to the viscera and were suppurative in nature. She had purulent meningitis, and the entire course of her illness was about 5 to 6 weeks.

The pneumonia was of the bronchial type as evidenced by the limited physical signs and x-ray findings. Multiple abscesses are more common in this latter type than in pure lobar pneumonia. The rather large size of the abscess in the left upper lobe makes it a likely source of the septicemia involving the endocardium as well as the other viscera.

SUMMARY

1. A case is reported presenting the association of two rare clinical pathologic entities, viz., acute bacterial mural endocarditis and acute interstitial pancreatitis.

2. The likelihood of the interstitial pancreatitis being the primary process is suggested.

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NODAL RHYTHM AND BUNDLE BRANCH BLOCK FOLLOWING ASPIRIN HYPERSENSITIVITY*

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DRUG sensitivity is not uncommon, and, as aspirin is a favorite drug, one would expect to see an occasional abnormal reaction after its ingestion, but Gardner and Blanton[1] have shown that although enormous quantities of aspirin are consumed every year in the United States, only a rare case of severe hypersensitivity has been reported.

Skin rash and gastrointestinal disorders are the common sequelae to drug overdosage; severe anaphylactic manifestations, such as dyspnea, cyanosis, asphyxia, generalized circulatory collapse, convulsions, or profuse urticaria, are very unusual. Wilcox and Andrus,[2] in animal experiments on the isolated heart, have provoked anaphylactic reactions using horse serum, in which the electrocardiogram showed ectopic rhythms, PR prolongations, and QRS and T changes. These abnormalities were transient and frequently disappeared within fifteen minutes.

Our case represents an analogous situation in the human heart. The sensitizing mechanism was a drug rather than horse serum. Anaphylactic shock with transient nodal rhythm and bundle branch block occurred after the ingestion of five grains of aspirin. Within twenty-four hours all of these phenomena had disappeared.

CASE REPORT

R. M., a white male, aged 50 years, was first seen in June, 1937, at the Medical College Hospital. He was brought to the hospital as an emergency, complaining of a sudden attack of breathlessness associated with pain in his right chest. He had suffered two previous attacks within a period of a month; both of these had subsided rather promptly after rest in bed for several hours. The pain in the chest was just to the right of the sternum at the fourth intercostal space and did not radiate. The examination at that time showed the blood pressure to be 135/85, temperature, 98.6° F., pulse, 80. An electrocardiogram was taken, but the only abnormality was a left axis deviation and a negative T wave in Lead III. The patient left the hospital two days after admission.

He was readmitted four years later in January, 1941, complaining again of breathlessness and pain in his right chest, but at this time it was associated with excessive lacrimation and a serous nasal discharge. The entire episode occurred after he had taken five grains of aspirin. The patient volunteered the information that he had noticed the same type of discomfort after taking aspirin in the past. The examination revealed a very weak male, bathed in perspiration, with chest pain which radiated to both arms. The skin was cold and clammy, pulse rate, 110, BP, 110/60, temperature, 100° F. An electrocardiogram was immediately taken, and this tracing showed a nodal rhythm and bundle branch block. The temperature subsided to normal in twenty-four hours. The urinalysis was negative, hemoglobin, 86 per cent Sahli, 4,900,000 erythrocytes, white blood cells, 13,400, with 89 polymor-

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phonuclear neutrophiles, 6 lymphocytes, and 5 monocytes; the Wassermann was negative. The next day all of his symptoms had subsided. Another electrocardiogram showed that the nodal rhythm and bundle branch block had disappeared; the only unusual change in the tracing was a negative T-4. The patient remained in the hospital five days and then signed his release.

His third admission to the hospital was on March 12, 1943. At that time he returned for an elective operation on his nose. An electrocardiogram revealed the same contours as the original tracing in 1937. The chart shows the electrocardiographic changes.

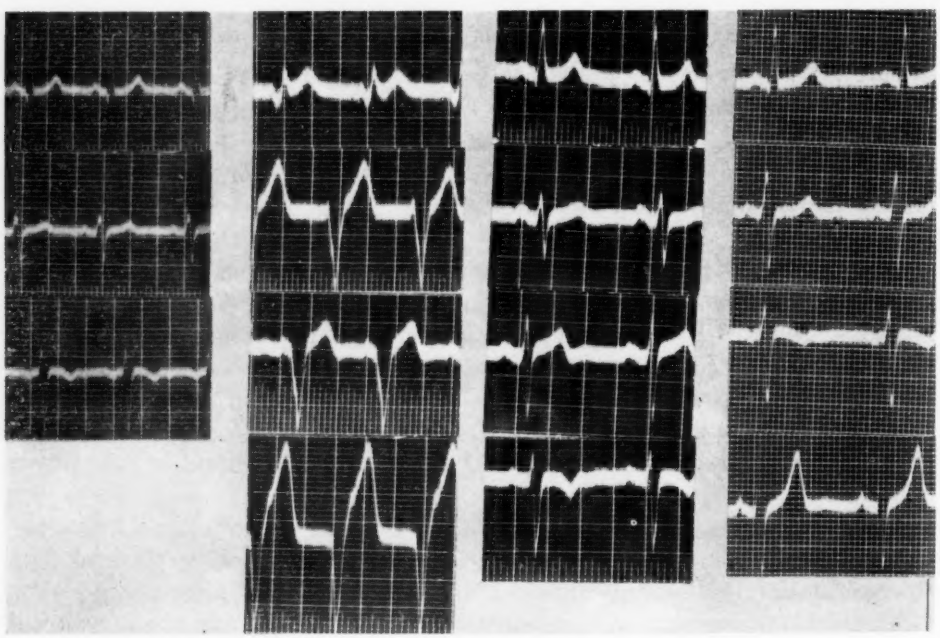

Fig. 1.—*A*, QRS—0.06; deep Q-1; deep S-2; negative P-3; deep S-3; negative T-3. Left axis deviation.

B, QRS—0.12; varying P-1; deep Q-1; slurred R-1; wide QS-2; high T-2; wide QS-3; high T-3; deep Q-4; high T-4. Nodal rhythm and left discordant bundle branch block, left axis deviation.

C, QRS—0.08; deep Q-1; deep S-2; deep S-3; diphasic T-3; negative T-4. Left axis deviation.

D, QRS—0.06; deep Q-1; deep S-2; negative P-3; deep S-3; negative T-3. Left axis deviation.

DISCUSSION

Although the original electrocardiogram taken in 1937 was not quite normal, the left axis deviation and T-3 negativity were not considered significant changes in this particular case, and after six years there were no added abnormalities in the electrocardiogram. Longcope[3] has demonstrated that there is a definite relationship between clinical serum disease and certain drug reactions. He considered the arsphenamines and sulfonamides the more dangerous drugs, but we have shown that aspirin may produce similar phenomena. Rich[4] studied the tissues of patients who had died after receiving horse or rabbit serum. The most interesting necropsy finding in these cases was an acute arteritis and periarteritis affecting the smaller vessels, and he concluded that vascular lesions of this type could be a manifestation of an anaphylactic form of hypersensitivity in man. We assume that this same type of reaction occurred in our case, but that it was transient and the patient was not left with any permanent damage of the smaller coronary vessels.

CONCLUSION

Aspirin is an excellent analgesic and, considering its enormous consumption, only occasionally causes any severe reactions in human beings. This case is an example of very unusual sensitivity to the drug.

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TRANSIENT T-WAVE INVERSION FOLLOWING PAROXYSMAL TACHYCARDIA*

S. L. ZIMMERMAN, MAJOR, M.C., A.U.S.

ABNORMALITIES of T waves of the electrocardiogram following bouts of paroxysmal tachycardia, unassociated with myocardial infarction including T-wave flattening and inversion, have recently been reported.[1, 2, 3, 6] Their occurrence may follow either supraventricular tachycardias or, probably more often, those of ventricular origin. The inversion may persist a varying length of time following cessation of the paroxysm, even as long as two months, without necessarily entailing a diagnosis of infarction. Geiger[2] suggests that there may be some relationship between the length of the attack and the duration of the T-wave inversion. This, however, has not been the usual experience.

The importance of correct diagnosis in these cases, especially with reference to myocardial infarction, appears obvious. It is believed that the following three cases illustrate the evolution of these T-wave changes clearly, and it is felt that their significance should be more generally recognized. The first case is one of supraventricular tachycardia; the second and third are of the ventricular type.

CASE REPORTS

CASE 1.—B. L., a 47-year-old white female, was admitted at 10:30 P.M. Aug. 4, 1943, with a paroxysmal tachycardia which began at 9 P.M. the same night. She gave a history of numerous attacks of rapid heart action, for a number of years, lasting from ten minutes to thirteen hours. During these attacks she had been cognizant of palpitation, but between them she was asymptomatic. No unequivocal history of a diminished cardiac reserve was obtained, and there was no history of retrosternal distress related to exertion. Her activities had been markedly restricted because of them. She had taken quinidine sulfate on occasions, and this drug had been quite effective in diminishing the number of paroxysms, as well as terminating them, once they had begun. She had chorea as a child, following an attack of tonsillitis. The remaining history was not relevant. Examination disclosed a 47-year-old white female, somewhat flushed, in the semiorthopneic position, with a regular ventricular rate of 200 to 210 per minute. The blood pressure was 130/110. The fundi revealed a mild retinal arteriosclerosis. The heart was not enlarged. There were no thrills, shocks, or abnormal thrusts. No murmurs were heard. The radial vessels were thickened. There were no evidences of congestive failure. The remaining examination was irrelevant. The electrocardiogram revealed a supraventricular tachycardia, with a rate of 210 per minute. (Fig. 1*A*.)

Orbital pressure, carotid sinus pressure, and vomiting resulted in some gradual slowing, but the rapid rate soon ensued, following cessation of stimulation. She was given dilaudid HCl $\frac{1}{32}$ grain. She had stated that she was sensitive to morphine, and was reassured, but her arrhythmia continued. At 12:55 A.M. Aug. 5, 1943, she was given 20 mg. of mecholyl. There was an intense vasodilatation, especially of the face and chest. The blood pressure fell to 112/90, but the rate remained unchanged. Carotid sinus pressure following mecholyl failed to alter the arrhythmia.

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Quinidine sulfate in doses of six grains at 1 A.M. and six more grains at 6 A.M. was followed by an abrupt change in rate at 8:20 A.M. the morning of August 5. The paroxysm lasted eleven hours and twenty minutes. A second electrocardiogram (Fig. 1*B*) showed a regular sinus rhythm with a rate slightly over 100, an inverted T-1 and T-4, and a flattened S-T interval with a shallow T wave in Lead II. The quinidine sulfate was continued in doses of three grains four times daily, and on Aug. 7, 1943, electrocardiogram (Fig. 1*C*) was obtained. The T waves in Leads I, II, and IV *F* reverted to an upright position. The patient was discharged and she resumed her usual activities immediately.

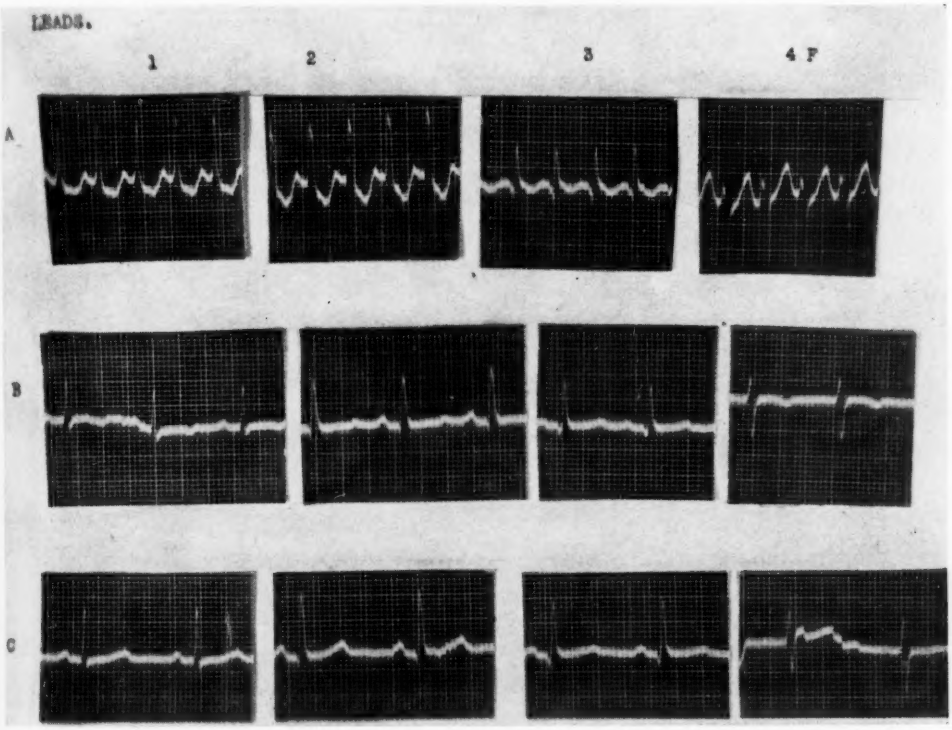

Fig. 1.

Comment.—A paroxysm of supraventricular tachycardia of eleven hours and twenty minutes' duration, followed by transient inversion of the T waves in Leads I and IV *F*, treated with sedation, vagal stimulation, and quinidine is presented. Two days following cessation of the arrhythmia, the T waves had reverted to their normal upright contour, in spite of continuation of the quinidine salt. There was no clinical or electrocardiographic evidence of organic heart disease, although the patient had a mild hypertension and a moderate degree of retinal and peripheral arteriosclerosis. That there was no myocardial infarction was obvious from the clinical course, the rapid reversion of the T-wave changes, and the absence of any S-T elevation and Q waves.

CASE 2.—B. W. M., a 46-year-old white male, was first admitted Feb. 12, 1939, complaining of nausea and a rapid, "fluttering" heart action, with sudden onset, the day prior to admission, accompanied by shortness of breath. There was a history of exertional dyspnea but none of anginal syndrome. Other than the above, his health had been considered good. There was no history of antecedent hypertension, syphilis, or rheumatic fever.

On admission, he was cyanotic and moderately dyspneic. The ventricular rate was 216 per minute and quite regular. The pulses were barely perceptible. The blood pressure was 90/70. There was no apparent enlargement. No murmurs were heard. The peripheral vessels

were sclerotic. There was no evidence of congestive failure. The remaining findings were not relevant. Carotid sinus pressure failed to alter the rate significantly. The electrocardiogram revealed a ventricular tachycardia (Fig. 2 *A*).

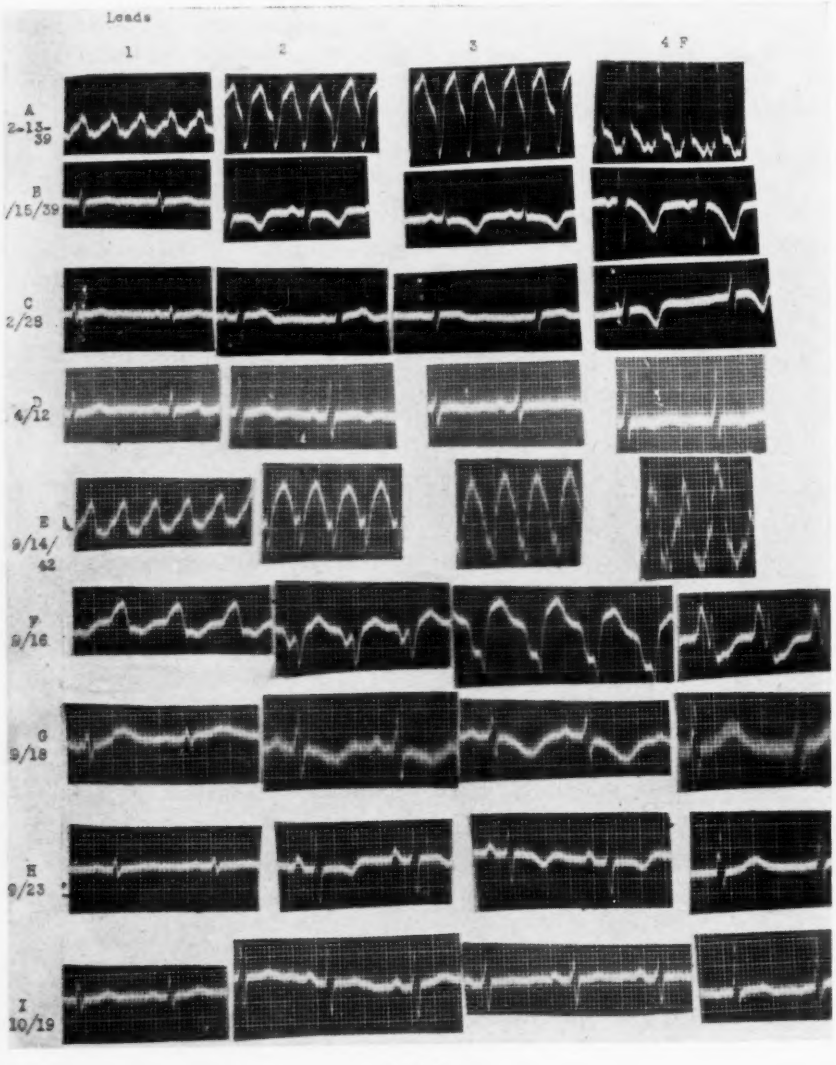

Fig. 2.

Course.—The patient remained afebrile throughout his hospital stay. No friction rub was audible. He was given small doses of digitalis, and the sinus rhythm became re-established on Feb. 15, 1939, the paroxysm lasting about four and a half days. Electrocardiogram (Fig. 2*B*), obtained Feb. 15, 1939, revealed marked inversion of the T waves in Leads II, III, and IV *F*, with depression of the S-T junction in Leads II, III, and possibly IV *F*. No quinidine was administered, but digitalis was continued in small daily doses, the patient probably having never been fully digitalized. The electrocardiogram of February 28 revealed a normal sinus rhythm, a diphasic plus-minus T-2 and T-3, and a deeply inverted T-4 (Fig. 2*C*), the rhythm continuing regular sinus. His course continued uneventful, and the last electrocardiogram during this admission (Fig. 2*D*) revealed an upright T-2, a very shallow, probably diphasic, plus-minus T-3, and a shallow, inverted T-4. The U waves were prominent in all leads. The patient was discharged as having attained maximum benefit, Apr. 16, 1939.

On the second admission, Sept. 14, 1942, he re-entered with an attack of paroxysmal tachycardia of 48 hours' duration, which had not responded to digitalis or quinidine administered by his physician. He was excited, apprehensive, dyspneic, and somewhat cyanotic. The hands were cold and clammy. The ventricular rate was 208 and regular. The blood pressure was unobtainable. There were no murmurs and the heart was not enlarged. There was no evidence of congestive failure. The remaining findings were not consequential. Further history this time revealed the fact that he had had numerous attacks of tachycardia since 1929, lasting from a few hours to a few days. Electrocardiogram (Fig. 2*E*) was obtained on admission.

Course.—The patient was afebrile throughout his hospital stay. No friction rub developed. He complained of no pain. Large doses of quinidine sulfate were started orally on the day of admission, augmented with prostigmine. At 9 A.M., Sept. 16, 1942, the rate was 112 per minute, after he had a total of 72 grains of quinidine sulfate. Electrocardiogram at this time revealed a persistence of the ventricular tachycardia, with a rate of 132 per minute (Fig. 2*F*). The QRS complexes were markedly widened. The patient felt better. On September 18, the ventricular tachycardia gave way to a normal sinus rhythm with a rate of 84 per minute. By this time he had 150 grains of quinidine sulfate. Electrocardiogram (Fig. 2 *G*) revealed marked inversion of the T waves in Leads II and III. The waves were large and broad. The QT interval was prolonged, and the ST junctions in these leads were depressed. The contours differed from the inverted T waves following the initial paroxysmal tachycardia (compare with Fig. 2 *B*), and, in addition, T-4 remained upright.

Quinidine intake was reduced, but still administered. On September 23, electrocardiogram (Fig. 2 *H*) showed a shallow T-1, with inverted T waves in Leads II and III, but not nearly so broadened as they were in the prior tracings. From then on, his recovery was uneventful. Quinidine was continued in dosages of 9 to 12 grains daily, and on October 18, his final electrocardiogram (Fig. 2 *I*) revealed upright T waves in all leads.

Comment.—The preceding case is one of recurrent bouts of tachycardia over a period of fifteen years, only two of which were studied and which proved to be of the ventricular type. It is believed that this patient had undoubted organic heart disease. The first paroxysm lasted four and a half days and terminated without the use of quinidine. This was followed by inversion of the T waves in Leads II, III, and IV *F*, with depression of the S-T junctions in the same leads. After a period of almost two months, the T waves became upright in Lead II, diphasic in Lead III. The second paroxysm lasted six days and was terminated after he had received 150 grains of quinidine sulfate as well as prostigmine, over a period of four days. Again the T waves progressed through a similar evolution, although differing in configuration and duration from those following the first attack, which had been uninfluenced by quinidine. T-4 did not become inverted following the second attack. After a period of one month, all T waves reverted to an upright position, and during this period quinidine had been continued in maintenance doses.

The question of myocardial infarction must be considered. However, it is believed that it did not take place because of the following reasons: There was no chest pain, and, other than the arrhythmia, there was nothing which suggested an infarct, clinically. Electrocardiographically, there were no reciprocal S-T deviations and no Q wave evolved. The S-T junctions were depressed, rather than elevated, in the leads showing T-wave inversion. The reversal of T waves occurred quite rapidly (one month following the second paroxysm), and the changes were very similar to those following the first paroxysm. Finally, the history of often repeated paroxysms of rapid heart action makes it appear unlikely that an occlusion with a myocardial infarct ushered in each of those attacks.

CASE 3.—A. H. M., a 48-year-old white male, was admitted Oct. 5, 1942, because of genitourinary difficulty. He had had hematuria, associated with the passage of gravel, for eight years prior to admission. Three weeks before, he had a chill, and this recurred daily and was associated with a temperature rise. He was treated for malaria at home. Associated with the chill, he noticed a "red" urine, which continued almost up to the time of admission. A day or so before entrance, he had a pain in his right flank which radiated back to the right costovertebral region.

For a number of years he had noticed precordial pain associated with exertion, sometimes requiring narcotics for relief. His electrocardiograms in the past had always been within normal limits. He was a known hypertensive, but his heart was never enlarged. There was no history of syphilis or rheumatic fever. He offered no complaints with reference to his cardiovascular system at the time of admission.

On examination, he showed a ventricular rate of 200 per minute. The rhythm appeared to be absolutely regular. Carotid sinus pressure did not alter it appreciably. There were no murmurs; the heart was not enlarged. Blood pressure was 112/96. There was no evidence of congestive failure; in fact, he appeared comfortable, neither dyspneic, orthopneic, nor cyanotic. The radial vessels were easily compressible, their walls thickened. Electrocardiogram (Fig. 3 *A*) was obtained on admission.

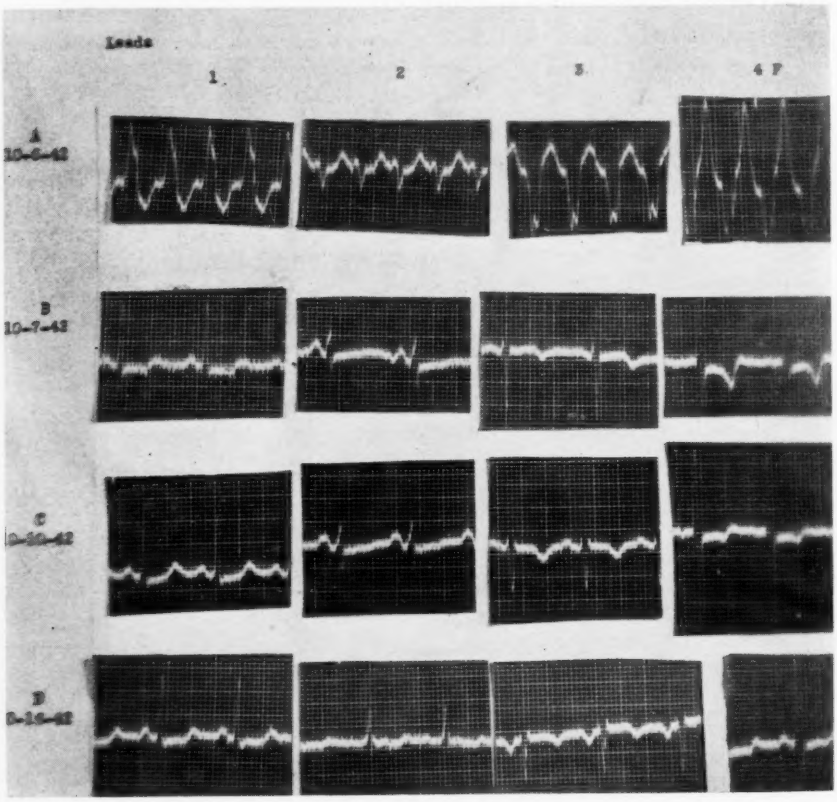

Fig. 3.

Course.—The patient had a low-grade rectal temperature, reaching a level of 99.8 degrees, which continued for a period of eleven days. The white blood count was 10,000, with 55 per cent polymorphonuclears, and 12 per cent eosinophiles. The sedimentation rate (Westergren) was 14 mm. per hour on October 8, and 2 mm. per hour on October 12. He did not complain of retrosternal or precordial pain.

With sedation, including morphia, the tachycardia ceased sometime during the night of October 6. No friction rub appeared. He felt well and offered no complaints. No quinidine had been administered. Electrocardiogram (Fig. 3 *B*), obtained after the establishment of a sinus mechanism, revealed depression of the S-T segments in Leads I and II, with a gradual ascent in Lead II and no reciprocal elevation in Lead III. There was no Q wave in the third lead. The T wave in Lead I was diphasic, that in Lead IV *F* was sharply inverted, with a depressed S-T segment. These findings did not fit any generally known infarction pattern, and a diagnosis of left lateral wall infarction was then entertained.

The sounds remained good. No friction rub appeared, and the blood pressure rose. The patient felt well. Quinidine therapy was then instituted, and on October 10, the electrocardiogram (Fig. 3 *C*) revealed an upright T-1, an isoelectric T-2, with a depressed S-T junction in both of these leads. T-4 inversion became more shallow and was giving way to diphasism. S-T-4 still remained depressed. On October 16, the tracing (Fig. 3 *D*) showed no essential changes in the limb lead with the exception of inversion of P-3, but T-4 was now upright, and there remained only minimal depression of the S-T junction in this lead. Quinidine had been administered in the same doses, after the second electrocardiogram was obtained, throughout his hospital stay. He refused urologic work-up and was discharged as asymptomatic soon thereafter. The cause for the eosinophilia was not established.

Comment.—The major T-wave changes occurred in Lead IV *F*, with lesser changes in Lead I, and the reversion to an upright position required but nine days following cessation of the ventricular tachycardia. There were no other electrocardiographic changes which might have unequivocally suggested an infarction. Moreover, there were no clinical findings to support such a contention. The S-T depression in Lead I remained, that in Leads II and IV *F* disappeared. No Q wave appeared. The S-T deviations were not reciprocal and were not of the type associated with a known infarction pattern. Moreover, there were no serial changes to support the original contention of a left lateral wall infarct.

DISCUSSION

The case of supraventricular tachycardia failed to respond to the usual methods, including mecholyl in the dosage given. It persisted over eleven hours and terminated abruptly. T-wave inversion in Leads I and IV persisted for a period slightly less than two days. There was no evidence of organic heart disease. The mechanism of T-wave inversion following tachycardia is, in general, not known. Whether the patient had an "occult" coronary sclerosis which became manifest with a relative cardiac anoxia as a result of the tachycardia is conjectural. Whether "myocardial fatigue" alone can cause this inversion, in the presence of an intact, sufficient coronary circulation, has not definitely been proved, although it has been suggested by some.[6, 8] That the T-wave changes did not result from the quinidine is probable, in view of the relatively small dosage and the rapid return to an upright position, even though quinidine administration had been continued. She had, also, on various occasions, taken quinidine in similar doses, with no effect on her prior electrocardiograms.

A rather important point in this respect is the relatively infrequent association of paroxysmal auricular tachycardia with myocardial infarction. In a large series of acute coronary occlusions, Rosenbaum and Levine[5] did not observe one case, although other arrhythmias were not uncommon. Master and his coworkers[7] reported three cases in a group of 300 patients. It, accordingly, would appear, from a statistical standpoint at least, that T-wave inversion following supraventricular tachycardias is usually not due to an associated infarct, although only careful observation, both clinically and electrocardiographically, may be necessary before any definite diagnosis can be made, especially if the T-wave inversion persists for a matter of weeks. The important point to remember is that such inversion does occur and may persist for a period as long as two months, in the absence of organic heart disease, not to mention myocardial infarction.

That the duration of the attack bears no relationship to the length of T-wave inversion is suggested by comparing the time required for the reversion of the

T wave in Case I with the case of Geiger's.[2] Both had a paroxysmal tachycardia of supraventricular origin, of comparable duration, and yet Geiger's patient had persistent T-wave inversion for twenty-six days, as compared with less than two days for this case.

When ventricular tachycardias result in T-wave abnormalities, the diagnostic problem with reference to ruling out coronary occlusions is much more difficult. Following the first paroxysm in the second case, such a diagnosis was entertained, in view of the long persistence of the T-wave inversion, a period of almost two months. Following the second paroxysm, of six days' duration, T-wave inversion persisted for only slightly more than one month. This again suggests that there does not appear to be any relationship between the duration of inverted T waves and the length of the causative paroxysm.

The reasons for not entertaining quinidine as the cause of the T-wave inversion in this and in the third case have been discussed previously mainly because similar inversion existed following both paroxysms in the second case, even though no quinidine had been given during or following the first attack, and because following the second attack, the T waves returned to an upright position during the administration of quinidine. In the third case no quinidine was administered during the attack. In fact, in our experience with ventricular tachycardias treated with massive doses of quinidine, T-wave inversion was the exception rather than the rule, unless it was part of the electrocardiographic evolution of a "protracted myocardial insufficiency."

The importance of proper evaluation of these electrocardiographic changes, from a therapeutic and prognostic standpoint, cannot be overemphasized. To lean toward the side of conservatism, with respect to therapy, is, on the whole, commendable, but the avoidance of erroneous diagnoses carrying ominous prognoses should be strived for. It is believed that cardiac invalidism has been enforced upon many patients because of failure to appreciate the possibilities of the mechanisms described.

The probability of an associated infarction must be considered etiological in these T-wave abnormalities if Q waves appear, if progressive changes in reciprocal S-T deviations are evolved (in occlusions the S-T intervals are elevated in those leads where the T waves become inverted) and if other clinical factors suggesting an infarct coexist. These changes probably do not occur in "benign," even though persistent, T-wave inversion associated with and following paroxysmal tachycardia.

SUMMARY

1. One case of supraventricular tachycardia and two cases of ventricular tachycardia followed by T-wave inversion in multiple leads, persisting for a variable period of time and not associated with myocardial infarction, are presented.

2. The importance of correct evaluation of these changes is stressed.

3. The role that quinidine played is discussed. It does not appear to have been of etiological importance.

4. Certain differential electrocardiographic findings are discussed, and their importance in excluding myocardial infarction is stressed.

5. Persistent inversion of T waves following tachycardias is not necessarily of ominous prognostic import.

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COTTON HOSE AS A VEHICLE FOR A FUNGICIDE IN TREATMENT OF ATHLETE'S FOOT

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GOOD results in the treatment of fungous infections of the hands and feet with copper sulfate driven into the skin by iontophoresis have been reported by Haggard, Strauss, and Greenberg.[1] Advantage over other means of treatment was attributed to the bringing of the fungicide into close contact with the causal agent. The acetate ion was recently found to be more fungicidal than the sulfate ion by Williams and Southworth.[2, 3]

The idea of impregnating cotton hose with a fungicide for use in the treatment of athlete's foot appeared to have merit, not only because hose are in close contact with the foot during waking hours, but also because treated hose bring the fungicide in contact with the shoe lining, which frequently is a source of reinfection. Some exploratory tests were, therefore, made, but unfortunately the work has now been interrupted and it is impossible to complete the study as originally planned. However, the results to date were so promising that they are reported here for whatever assistance they may be to other investigators.

EXPERIMENTAL

Selection of Fungicide.—The experimental work was begun using a five per cent solution of copper sulfate for impregnating the cotton hose. In warm weather especially, this concentration, although reducing the effect of the fungi, proved irritating to the skin of some of the subjects. In one instance, a lower concentration was therefore used. In later work, copper acetate was substituted for copper sulfate in cases not responding satisfactorily.

Impregnation of the Hose With Copper Salts.—Clean, dry, cotton socks of good grade ("peds" for the women) were soaked for 30 minutes in the copper salts solution at 40° centigrade. They were then put through an ordinary clothes wringer set loosely but giving enough pressure that the wrung hose, although still wet, did not drip and the solution was evenly spread throughout the hose. (The set screws of the wringer were always turned until the second thread from the top of the screw touched the crossbar.) After drying, the hose were ready for wear.

Subject and Procedure.—The subjects cooperating in the study were 17 men and one woman, each of whom was afflicted with athlete's foot. The initial examination and the diagnosis for 12 subjects were made by a physician. The first examinations of the other persons and all of the periodic examinations at intervals of approximately one month were made by one of the authors (P. J. C.).

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TABLE I

RESULTS OF TREATMENT OF ATHLETE'S FOOT WITH VARIOUS CONCENTRATIONS OF COPPER SALTS IMPREGNATED IN COTTON HOSE

	DURATION OF INFECTION	DURATION OF TREATMENT	RESULTS
Copper sulfate (5 per cent):			
Severe (Interdigital desquamation):			
B. B.*	15 years	4 months	Improvement
C. M.*		2 months	Some improvement
N. S.	10 days	1.5 months	Appeared to be healed
Moderate (Fissures and scaling between toes):			
M. C.*	6 years	6 months	Skin condition worse with desquamation. (History of streptococcic infection)
I. O.*	6 years	3 months	Appeared to be healed
Mild (Fissures between toes):			
L. S.*	As long as he can remember	6 months	Improvement
G. Z.*	15 years	5 months	Appeared to be healed
H. H.*	10 years	4 months	Improvement
E. G.*	7 years	7 months	Improvement
K. H.*	7 years	2.5 months	Improvement
J. B.*	3 years	5 months	Improvement
A. D.*	2 weeks	4 months	Appeared to be healed
Copper sulfate (5 per cent) superseded by copper acetate (5 per cent):			
Moderate (Scaling and fissures between toes):			
S. H.	20 years	3 mo., 5 per cent sulfate	Improvement but complained of general foot skin irritation
		1 wk., 2½ per cent sulfate	
		7 wk., 1 per cent sulfate	
		2 mo., 5 per cent acetate	Appeared to be healed
P. A. B.	9 years	3 mo., sulfate	Intermittent improvement
		4 mo., acetate	Improvement
Q. C.—Desquamation between toes; soaked feet 10 minutes at night in 1 per cent copper acetate for 5 months. Hammer toes	2 years	5 mo., sulfate	Not improved
		5 mo., acetate	Improvement
T. B.—Feet deformed due to poliomyelitis	1 year	3.5 mo., sulfate	Intermittent improvement
		2.5 mo., acetate	Improvement
Mild (Fissures between toes):			
D. H.*—Reinfection. Some interdigital desquamation between toes	5 years	5 mo., sulfate	Appeared to be healed; later reinfected
		1 month, acetate	Appeared to be healed
Copper sulfate (2½ per cent) superseded by copper acetate (2½ per cent):			
Moderate (Vesicles on heels: erythema, scaling between toes):			
U. M.	8 years	3 mo., sulfate	Some improvement
		2 mo., acetate	Greater improvement

*First examination made by physician. Sincere appreciation is expressed to Dr. J. D. Silay of Greenbelt, Maryland, for his cooperation.

Each subject was furnished with clean treated hose as needed and was instructed to wear a clean pair each day, reporting any unpleasant sensations. After each day's wearing, the hose were returned to the laboratory where they were laundered, mended, and reimpregnated. The soiled socks were soaked in tap water at 40° centigrade for 30 minutes, rinsed, soaked again in tap water at 70° centigrade for 30 minutes for the destruction of the fungi responsible for athlete's foot. They were then washed in soapsuds, rinsed, dried, and impregnated with the copper salts.

RESULTS

Table I shows the kind and concentration of copper salts used, a brief description of the skin condition at the beginning of the period of treatment, the duration of infection prior to treatment, the length of time the socks impregnated with copper salts were worn, and the skin condition at the close of the study.

The skin of 6 subjects appeared to be normal at the close of the period of observation. That of 11 others was improved. The skin condition of one (M. C.) became worse. It is believed that the condition of two of these (B. B. and C. M.) might have shown further improvement had the subjects been more conscientious in wearing the socks. Complaints of irritation were not made when the acetate was used, but one subject out of six objected to the odor. The acetate seemed to be more beneficial than the sulfate. Itching was usually relieved within a week. No harmful effect on the shoes was reported.

SUMMARY

Eighteen subjects with clinical athlete's foot wore cotton hose impregnated with copper sulfate or acetate for periods of 1½ to 10 months. The skin of six appeared normal, eleven showed improvement, and one was worse at the end of the experiment. The acetate seemed to be more beneficial than the sulfate.

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THE EFFECT OF HEAT PRODUCED BY SHORT WAVE DIATHERMY ON THE ACTIVITY OF MUSCLE*

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INTRODUCTION

ALTHOUGH some form of heat has been employed since earliest times in the treatment of pain, injury, and weakness of skeletal muscle, the problem of its use is perplexing because there is incomplete scientific evidence as to what effect heat has on the intact muscle. The problem is further complicated by the variety of methods employed for applying heat and the resulting attempt to evaluate the efficiency of each. Since short wave diathermy is a form of heating widely used in the treatment of muscle, the experiment reported here was undertaken in an attempt to find out how its application affects the duration of the various phases of muscular contraction and to what degree each phase is affected.

An examination of the literature yields but little experimental data concerning the effects of the application of heat on the response of intact muscle.

Denkman[1] studied the effect of heat generated by the infra red lamp (radiation) on the response of the intact gastrocnemius muscle. Twenty-four subjects were used. Her conclusions were that after twenty minutes of radiation there was a shortening of the latent and contraction periods of muscular activity. No definite conclusions were drawn from her data on relaxation time.

Tuttle and Williams[2] studied the effect of autocondensation and high frequency currents (conversion) on the tonus of skeletal muscle. The extent of the knee jerks of nine subjects, before and after heating, was compared. It was found that the autocondensation current when applied in sufficient quantities to cause perspiration reduces the extent of the reflex. The increase in temperature evidently causes a relaxation of the muscles and thus a decrease in tonus.

Other experiments pertinent to the subject of the effects of short wave diathermy on muscle are reviewed by Bierman.[3]

This experiment was conducted on twenty normal individuals, sixteen women and four men, between the ages of twenty and forty years. The group was selected at random. Each individual's muscular response was recorded before rest and after twenty minutes of rest. On the following day at the same hour his muscular responses were recorded before heat and after twenty minutes of application of heat with short wave diathermy. The data on the effects of rest were collected so as to be sure that any effects appearing in the heat records could not be attributed to rest. The contraction of the intact gastrocnemius muscle was obtained by applying an electric stimulus to the motor point.

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The Stimulating Unit.—The source of the stimuli was the laboratory type of inductorium. One lead from the secondary winding of the inductorium was terminated in a copper electrode ¾ of an inch in diameter, which was covered with electrode jelly. This electrode was strapped over the medial motor point of the gastrocnemius muscle. The other pole of the secondary winding was terminated in a metal electrode with a surface area of 6 by 6 inches. This electrode covered with wet Canton flannel was placed on the outer part of the thigh. The primary winding of the inductorium was placed in series with a hand switch, two dry cells, and a signal magnet. Submaximal break induction shocks were used as stimuli.

The Recording Device.—The data were recorded on the smoked paper of an extension kymograph.

The subjects were placed prone on a table. A stout string was attached to the sole of the subject's shoe by a thumbtack. The string extended under the table and to a recording stylus suspended from a rubber band. Uniform tention was maintained on the string at all times by means of the rubber band. The time of the application of the stimulus was marked on the record by a signal magnet placed in the primary circuit. An electrically driven tuning fork vibrating at the rate of 100 double vibrations per second was placed between the signal magnet and the stylus attached to the subject's foot.

After the electrodes were applied, the tension on the muscle adjusted, and the recording device ready, the drum was spun, during which time the stimulus was delivered and the response recorded. Since all the recording styli were on the same ordinate, the speed of the kymograph was immaterial except that it was fast enough so that the time could be read to the nearest quarter of a double vibration. The greatest number of complete contractions which it was possible to record on one drum was recorded each time. The number of recordings found in the data were those which could be read with confidence as to validity.

The Heating Unit.—The portable, short wave diathermy machine used was an "Intratherm" Model P125, manufactured by the Therapeutic Oscillator Corporation, West Des Moines, Iowa.

The Record.—Records were made as stated in the section on the recording device. The first records were those of the response of the muscle before rest. The subject was allowed to rest for twenty minutes and then records were made of the response of the muscle. The subject returned the next day at the same hour and a record was made of the response of the muscle before heating. Then the electrodes from the inductorium were removed and a cuff condenser electrode was tied around the leg just below the knee. The other cuff condenser electrode was tied around the leg just above the ankle. The subjects were free to move the leg throughout the heating period. They were told to inform the investigator if the leg felt too hot all over, too hot in any one spot, or if there was a sensation of muscle cramping. The desired sensation was one of comfortable warmth. The current was turned on and left on for twenty minutes. The intensity of the current was regulated according to the subject's heat tolerance. After the heating period, the heating electrodes were removed, the electrodes from the inductorium replaced, and a record of the response of the muscle was recorded.

Reading the Record.—The technique of recording required that the response of the gastrocnemius muscle be represented as a downward movement on the smoked drum. However, for purposes of computation, the records were turned over and read from right to left. They are represented in this manner in Fig. 1. The top line is made by the recording stylus attached to the foot, the lower line by the signal magnet, and the middle one by the 100 double vibrations tuning fork. The procedure for calculation consisted of dropping an ordinate through the break in the signal magnet line, then one through the point at which the recording stylus left the base line. In order to establish a

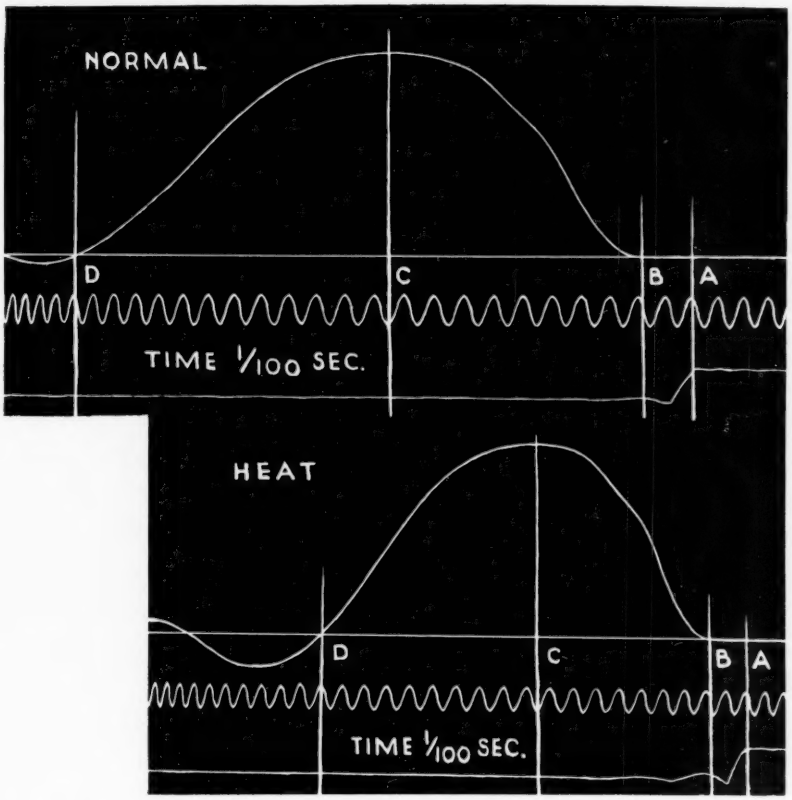

Fig. 1.—Record of the effect of heat produced by short wave diathermy on the activity of muscle. The upper figure shows the normal muscular response. The lower figure shows the muscular response after heat. Reading from right to left:

Normal		
A-B	Latent period	0.0175 sec.
B-C	Contraction period	0.0875 sec.
C-D	Relaxation period	0.1250 sec.
Heat		
A-B	Latent period	0.0175 sec.
B-C	Contraction period	0.0775 sec.
C-D	Relaxation period	0.0950 sec.

base line, the recording stylus line, before contraction occurred, was continued through the record. An ordinate was dropped through the middle of the flattest part of the top of the contraction curve, and finally an ordinate was dropped through the time line from the point where the recording stylus returned to the base line. It is evident that the latent time is the time elapsing between the

stimulus and the leaving of the base line by the recording stylus. The contraction time began at the place where the stylus left the base line and ended at the middle of the flattest part of the curve. The relaxation period began at the end of the contraction time and ended at the point where the stylus returned to the base line.

The duration of each period of the form curve was determined in seconds by reading the number of tuning fork vibrations between ordinates.

The Data.—A summary of the data relative to the effect of rest on the activity of the intact muscle is presented in Table I. The data show that no significant changes in the activity of the muscle were caused by a period of rest.

TABLE I

THE EFFECTS OF REST ON MUSCULAR ACTIVITY

PERIODS OF MUSCULAR ACTIVITY	GROUP MEANS		DIFFERENCES	S.E. DIFF.	t*	PER CENT CHANGE
	BEFORE	AFTER				
	sec.	sec.	sec.			
Latent	.0196	.0191	−.0005	.0006	0.8	2.6
Contraction	.1020	.1012	−.0008	.0014	0.6	0.8
Relaxation	.2464	.2546	.0082	.0046	1.8	3.3
Total time	.3680	.3700	.0020	.0067	0.3	0.5

*Differences are not significant at the 5 per cent level of confidence unless t is 2.1 or more.

Table II shows the effect of heat produced by short wave diathermy on the various phases of muscle activity. Each period of the form curve was significantly shorter after the muscle was exposed to heat. The greatest shortening effect occurred in the relaxation period. This conforms to the generally accepted idea that whatever alters the duration of the periods of the form curve produces the most pronounced effect on the period of relaxation.

TABLE II

THE EFFECTS OF HEAT ON MUSCULAR ACTIVITY

PERIODS OF MUSCULAR ACTIVITY	GROUP MEANS		DIFFERENCES	S.E. DIFF.	t*	PER CENT
	BEFORE	AFTER				
	sec.	sec.	sec.			
Latent	.0196	.0158	−.0037	.0009	4.1	19.0
Contraction	.1009	.0893	−.0116	.0033	3.5	11.5
Relaxation	.2543	.1976	−.0567	.0077	7.4	22.3
Total time	.3746	.3027	−.0719	.0073	9.8	19.2

*Differences are significant at the 1 per cent level of confidence when t = 2.9 or more.

It is of interest to compare the effects of cooling obtained by Tuttle[4] with those of heat reported here. An ice pack applied to the gastrocnemius muscle for 20 minutes prolonged its activity twice as much as the application of heat for 20 minutes shortened it. Obviously, this is due to the fact that the temperature of the muscle was lowered by the ice considerably more than its temperature was raised by short wave diathermy.

CONCLUSIONS

The effect of the application of short wave diathermy to the intact gastrocnemius muscle was investigated. The data show that all periods of the form curve of contraction were significantly shortened by the application of heat.

Although all periods were significantly shortened, the period of relaxation was changed most.

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THE EFFECT OF ARTIFICIALLY INDUCED FEVER ON ANAPHYLACTIC SHOCK IN ACTIVELY SENSITIZED GUINEA PIGS*

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In Collaboration With
W. M. Simpson, M.D.,† H. W. Kendell, M.D.,† and D. L. Rose, M.D.†

DeKRUIF and Simpson[1] have recently pointed out the lack of an explanation for the favorable effect of fever on certain unrelated diseases thought to be of an allergic nature. These include chorea and other manifestations of the rheumatic state, intractable asthma of an allergic type, and inhibition of dermatitis following injections of arsenicals in arsenic-sensitive patients being treated for syphilis. Gernez and Eloire[2] studied the effect of fever on a small series of serum-sensitive guinea pigs and found that they were temporarily desensitized at the height of fever and that the higher the fever the greater the protection afforded. Hyperpyrexia was induced electrically by short waves, a temperature rise of six to seven degrees Fahrenheit being induced in 10 to 15 minutes. Mirsky and Wassermann in collaboration with Goldman[3] fevered serum-sensitive guinea pigs by placing them in a heated fever box for 10 to 15 minutes until their rectal temperatures were 109 to 110° F., and upon injection of a fatal dose of serum at the height of fever they found that the guinea pigs were desensitized. In both of the experiments reported, the animals' temperatures were raised very rapidly. Since clinical experience has shown that the technique by which the fever is administered very definitely affects the individual's reaction during the treatment,[4] we first developed a procedure which was entirely safe for normal animals and which simulated that used for producing febrile temperatures in human subjects. This phenomenon of desensitization by hyperpyrexia in serum-sensitive guinea pigs was then reinvestigated.

METHODS

Groups of 10 to 20 albino guinea pigs of an inbred strain were sensitized by subcutaneous injection of 1.0 c.c. of a 1:100 dilution of normal horse serum. These animals were sufficiently sensitive 23 to 136 days after the sensitizing dose had been given. Both sexes were used, the age at the time of fevering being 12 to 25 weeks and the weight 575 to 915 grams. These mature animals were chosen because they were found to be less excitable and consequently easier to fever and inject. The serum was injected into a marginal ear vein in both the fevered and control guinea pigs. Immediately before using a group of

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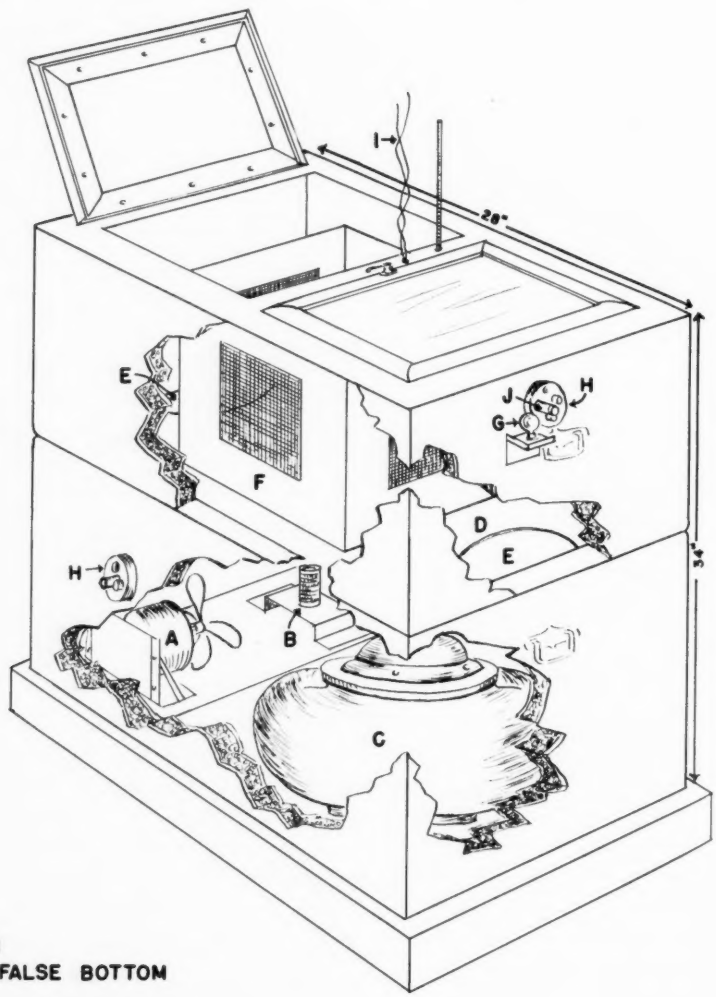
animals, the amount of serum fatal for 80 to 100 per cent of these animals was established. Generally five to eight animals were required for this titration. This amount of serum was then used as the assaulting dose for each fevered animal and its unfevered control. The assaulting dose of serum, therefore, varied from experiment to experiment, and if two groups of animals were used the dose sometimes varied within an experiment.

For induction of fever, hyperpyrexia induced by short waves was found to be unsatisfactory, since it was difficult to follow the rise in the animal's temperature because the thermometer had to be removed from the rectum during radiation. A large humidified cabinet related in principle to the hypertherm developed at the Kettering Institute for Medical Research for the production of hyperpyrexia in human beings was then tried. The animal was fastened to a modified Latapie board, the thermocouple inserted into the rectum, and the animal pushed into the cabinet. The temperature of the cabinet was then increased at a rate which did not raise the rectal temperature more than 1.0° F. during any five-minute period. This method was found to be satisfactory, although struggling of some animals occasionally led to an accelerated rise in their body temperature.

The method found simplest and most satisfactory was to place the guinea pig in a small air-conditioned cabinet, illustrated in Fig. 1.* A humidifier maintained the humidity of the cabinet at a point approaching saturation. The copper-constantan thermocouple, made with No. 30 B. and S. gauge wire, was prepared as described by Robinson.[5] Either rectal or subcutaneous temperature measurements were made. For rectal temperature measurements the couple was inserted into the rectum through a 2 mm. glass tube to a depth of four to six cm. The tube was then withdrawn by sliding it up the wire, care being taken to keep the couple in place in the rectum. The wires were fastened to the guinea pig with adhesive tape to prevent the couple from slipping out. The animal was allowed freedom of movement in a small cage within the cabinet. For subcutaneous temperature measurements a 1½-inch 19 gauge needle was pushed through the skin to a depth of about three cm. It was removed immediately, and the couple was inserted in its place to a depth of two to three cm. The couple was then fastened to the animal with adhesive tape, and the animal was placed in the cage within the cabinet.

After the guinea pig was placed in the cabinet, the glass-windowed doors, permitting observation of the animal, were closed, and the heat and humidifier were turned on. The cabinet temperature was raised to from 96 to 102° F. The dry heat regulated by the rheostat was adjusted to produce a slow increase, or a constant temperature, as found necessary to give the desired degree of hyperpyrexia. For maintaining the animal at the desired temperature, the cabinet temperature was usually lowered several degrees by removal of the rubber stoppers from the ventilators. The humidifier maintained the humidity close to 100 per cent of saturation throughout the fever session. Trials with normal animals preceded each experiment in which a different temperature was studied. This was done to determine the best method for regulating the cabinet

*The humidifier and heating device was installed by Mr. Edwin Sittler, Liebel-Flarsheim Co., Cincinnati, Ohio, formerly associated with the Kettering Institute for Medical Research.



KEY

A - FAN
B - HEATER
C - HUMIDIFIER
D - FALSE BOTTOM
E - AIR VENTS IN FALSE BOTTOM
F - ANIMAL CAGE
G - 15 W. BULB FOR I.V. INJECTION
H - VENTILATORS ADJUSTABLE WITH RUBBER STOPPERS
I - THERMOCOUPLE LEADS
J - 1 1/4" DIAMETER BRASS TUBE

Fig. 1.—Diagramatic drawing of the small animal cabinet used for producing hyperpyrexia in guinea pigs.

temperature to obtain satisfactory induction and maintenance of fever. Too rapid a rise in the rectal temperature caused by too high a cabinet temperature and sudden changes in cabinet temperatures were avoided. Cabinet and rectal temperatures were recorded at five-minute intervals. It was not possible to predict what cabinet temperature would raise an animal's temperature to a certain height, since individual guinea pigs varied somewhat in their response to temperature and humidity. Typical fever curves obtained by the use of this method are given in Fig. 2.

After the desired degree of hyperpyrexia had been attained and maintained for the intervals specified in Tables I, II, and III, an assistant quickly

removed the animal, placed its nose and mouth in a short brass tube 1¼ in. in diameter, leading into the cabinet. This precaution was taken to prevent the animal from breathing the cooler air of the room during injection. The injection was made into an ear vein, and the animal was returned to the cabinet immediately. A 15 watt bulb fastened to the cabinet just below the guinea pig's ear facilitated injection. Removal of the animal from the cabinet, injection, and return to the cabinet usually required less than three minutes. The injection

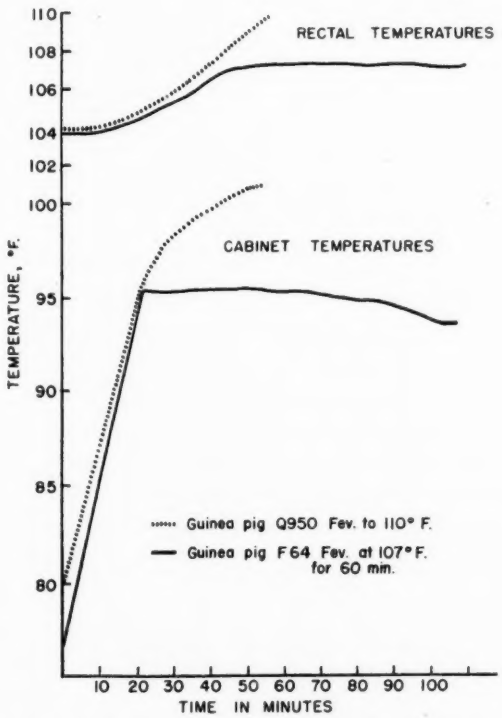


Fig. 2.—Rectal and cabinet temperatures during fever treatment of guinea pigs.

was made with a 0.5 or 1.0 c.c. syringe and a short leveled 27 gauge needle. Five minutes after the injection was made, the cabinet doors were opened and defervescence was allowed to take place spontaneously. Thirty to sixty minutes later the animal was transferred to its cage. Those animals that recovered lost weight for from 48 to 72 hours, but usually attained their prefever weight within a week.

The arbitrary method of recording the severity of anaphylaxis was as follows:

Severe: Severe respiratory distress in which the animal coughed, scratched its nose, hunched up, and gasped for breath. Cyanosis was marked, and the animal was prostrated. This was followed by death or recovery.

Moderate: Respiratory distress of shorter duration in which the animal coughed and scratched its nose but was not prostrated. Cyanosis was slight or absent.

TABLE I

THE EFFECT OF ELEVATIONS OF TEMPERATURE ON ANAPHYLACTIC SHOCK IN GUINEA PIGS

EXPERIMENT	FEVERED ANIMALS									UNFEVERED CONTROL ANIMALS								APPROXIMATE AGE IN WEEKS
	FEVER IN DEGREES FAHRENHEIT	NO. OF ANIMALS	DIED		RECOVERED	SEVERITY OF SHOCK				NO. OF ANIMALS	DIED		RECOVERED	SEVERITY OF SHOCK				
			< 1 HR.	1-24 HR.		SEVERE	MODERATE	SLIGHT	NONE		< 1 HR.	1-24 HR.		SEVERE	MODERATE	SLIGHT	NONE	
1	108°	5	3		2	3	2			5	5			5				12
2	108°	5	4		1	5				5	4		1	5				22
3	109°	5		3	2	2	1	1	1	5	5			5				13
4	110°	5			5		3		2	5	4		1	5				14
5	110°	5		2*	3	1	2	2		5	3		2	4		1		21
6	110°	5		1	4			5		5	4		1	4	1			16
7	110° and inj. with saline	5			5				5									20
8	109° to 111.2° Goldman method	7		5	2		3	1	3	7	6		1	7				25

*One of these animals died 2½ days after administration of horse serum.

Slight: The fur was ruffled; slight respiratory symptoms were evident as indicated by scratching the nose or sneezing. The animal was usually excited. Cyanosis and coughing were not observed.

EXPERIMENTAL

Five sensitized guinea pigs were artificially fevered to rectal temperatures of 108° F., and each was injected with a dose of horse serum lethal for 80 to 100 per cent of the control unfevered animals. In this experiment the animals were restrained as previously described. As shown in Table I, exp. 1, three animals which developed severe symptoms of anaphylaxis died within four minutes, while two developed moderate symptoms of anaphylaxis and recovered. The five control animals died in less than five minutes. When five animals were fevered to 108° F. but were not restrained, the same number of deaths occurred in the fevered and the control groups (Table I, exp. 2). These experiments show that little protection is afforded by a fever temperature of 108° F. when a fatal dose of serum is given as soon as this temperature is attained.

Five sensitized guinea pigs were then fevered to rectal temperatures of 109° F. and immediately injected with a dose of horse serum lethal for the control unfevered animals. Table I, exp. 3, shows that in this group in which the animals were restrained during induction of fever, none of the animals died of acute anaphylactic shock, although three died 3 to 24 hours later and two recovered. These results indicate that this temperature affords some protection against acute anaphylactic shock. In this and later experiments no correlation was found between the severity of shock and death 1 to 24 hours later.

Three groups of sensitized guinea pigs were then fevered to 110° F., and as soon as this temperature was attained a lethal dose of horse serum was injected. In these and subsequent experiments the animals were not restrained. In experiment 4 subcutaneous temperature measurements were taken, while in experiments 5 and 6 rectal temperature measurements were made. In these three experiments in which a total of 15 animals were used, only one animal showed severe symptoms; five showed moderate, seven slight, and two no symptoms of shock, indicating that a certain degree of protection is also afforded at this temperature. There seemed to be no significant difference in protection, whether the temperature was taken by the subcutaneous or by the rectal route. In another experiment subcutaneous and rectal temperatures were compared in four guinea pigs during induction of fever. The results which are not given in detail indicated that after a rectal temperature of 106° F. was attained, the subcutaneous temperature in three animals was from 1.5 to 2.0° lower than the rectal temperature. In the other animal the subcutaneous and rectal temperatures were equal throughout the induction period.

An attempt was then made to determine whether deaths from 1 to 24 hours following administration of serum were due to fever. Five sensitized guinea pigs were fevered to 110° F. and at the height of fever were injected with saline. As shown in Table I, exp. 7, none of these animals died, indicating that the late deaths were probably due not to fever but to anaphylactic shock or anaphylactic shock superimposed on fever.

TABLE II

THE EFFECT OF ELEVATIONS OF TEMPERATURE WHEN THE SHOCK DOSE WAS ADMINISTERED AT VARYING INTERVALS AFTER TEMPERATURE HAD BEEN ATTAINED

EXPERIMENT	FEVERED ANIMALS										UNFEVERED CONTROL ANIMALS								APPROXIMATE AGE IN WEEKS
	FEVER		NO. OF ANIMALS	DIED		RECOVERED	SEVERITY OF SHOCK				NO. OF ANIMALS	DIED		RECOVERED	SEVERITY OF SHOCK				
	DEGREE FAHRENHEIT	DURATION IN MINUTES		< 1 HR.	1-24 HR.		SEVERE	MODERATE	SLIGHT	NONE		< 1 HR.	1-24 HR.		SEVERE	MODERATE	SLIGHT	NONE	
1	109°	30	5		3	2			5		5	5			5				17
2	108°	30	5		1	4	1	1	1	2	5	5			5				17
3	108°	30	5		2	3		2	1	2	5	4		1	5				16
4	107°	60	5		3	2		3	2		5	4		1	5				16

TABLE III

THE EFFECT OF THE AGE OF ANIMALS ON THE RESPONSE TO AN ASSAULTING DOSE OF SERUM ADMINISTERED AFTER A FEVER TEMPERATURE OF 108° F. HAD BEEN MAINTAINED FOR 30 MINUTES

EXPERIMENT	FEVERED ANIMALS										UNFEVERED CONTROL ANIMALS								APPROXIMATE AGE IN WEEKS
	FEVER		NO. OF ANIMALS	DIED		RECOVERED	SEVERITY OF SHOCK				NO. OF ANIMALS	DIED		RECOVERED	SEVERITY OF SHOCK				
	DEGREES FAHRENHEIT	DURATION IN MINUTES		< 1 HR.	1-24 HR.		SEVERE	MODERATE	SLIGHT	NONE		< 1 HR.	1-24 HR.		SEVERE	MODERATE	SLIGHT	NONE	
1	108°	30	6	2	1	3	4	2			6	5		1	6				7
2	108°	30	8			8	1	1	4	2	8	7		1	8				112

The method of Mirsky, Wassermann, and Goldman[3] was then tried, in which method the body temperature was elevated very rapidly. The humidified cabinet was heated to from 111 to 112° F., and the animals were placed in it for 15 minutes. At this time their rectal temperatures varied from 109 to 111° F. A lethal dose of horse serum was then given. As shown in Table I, exp. 8, none of these guinea pigs died of acute anaphylactic shock, although five of the seven animals died within 24 hours after receiving the assaulting dose of serum.

A group of five guinea pigs was then subjected to a fever temperature of 109° F. and maintained for 30 minutes to determine whether better protection was afforded by maintaining them at this temperature for 30 minutes than by merely bringing them to this temperature and injecting the specific antigen immediately. The data given in Table II, exp. 1, indicate that maintenance of this temperature for 30 minutes affords slightly better protection than if the injection of serum is made as soon as 109° F. is reached.

To ascertain whether sensitized guinea pigs were protected by longer treatment at temperatures at which protection was not afforded by merely bringing them to these temperatures before injection, two groups of guinea pigs were fevered at 108° F. for 30 minutes and another group at 107° F. for 60 minutes. In these series shown in Table II, exp. 2, 3, and 4, none of the animals died of acute anaphylactic shock, although one to three of each group died within 1 to 24 hours after receiving a dose of horse serum lethal for 80 to 100 per cent of the controls. It appears that if the animals are held for 30 minutes at 108° F. or for 60 minutes at 107° F., the same protective effect is obtained as if they are brought to temperatures of 109 or 110° F. and the specific antigen injected immediately.

In induction of fever in human subjects the fluid balance is maintained by administration of large quantities of 0.6 per cent saline and other fluids, but unfortunately most guinea pigs refuse water or food during fever treatment. Five guinea pigs were therefore injected with 4.0 c.c. of normal guinea pig serum intraperitoneally immediately before being fevered at a temperature of 107° F. for 60 minutes. In this experiment two animals died within 10 minutes, one died 1 to 24 hours after administration of horse serum, and two recovered, indicating that injection of normal guinea pig serum previous to fever treatment did not eliminate shock or late deaths.

Since the age of the guinea pigs in the former experiments varied considerably, an experiment was made in which desensitization by artificially induced fever was compared in young and in old guinea pigs. These animals were fevered to rectal temperatures of 108° F., and this temperature was maintained for 30 minutes, after which a lethal dose of horse serum was injected. The age of the young group, composed of four males and two females, was seven weeks, and the age of the old animals, composed of six females and two males, was approximately 112 weeks. The results given in Table III show that young animals are much more difficult to protect than old ones. Four of the young animals showed severe symptoms and two showed moderate anaphylactic symptoms, while in the older group, one showed severe, one moderate, and four slight symptoms of shock, while two were completely protected. Two of the young

animals died within four minutes and one within 24 hours, whereas none of the older animals succumbed. The lethal dose of serum for the young animals was from 0.075 to 0.1 c.c., and for the older group it was from 0.35 to 0.75 c.c. It appears, therefore, that older animals do not become very sensitive and are easily desensitized, while young animals become very sensitive and are also more difficult to desensitize.

Most of the fevered and control animals that died within 1 to 24 hours were autopsied. The gross findings revealed marked congestion of the peritoneal wall, intestines, and stomach. In most cases the liver, spleen, kidneys, and lungs did not appear abnormal. A study of histologic sections of these tissues by one of us (H.E.C.) indicated that there was no essential difference between the findings in fevered and unfevered animals, except for a larger amount of lymphoid tissue in the lungs of all of the fevered animals dying within 12 hours. In general, significant histologic changes were confined to the lungs, kidney, liver, and spleen. In animals dead within 1 to 24 hours, varying degrees of extravasation of blood into the pulmonary alveoli and into the parenchyma of the medullary portion of the kidney, the liver, and the spleen were seen. Occasionally there were cuff hemorrhages in the cortex of the brain and some hemorrhage in glomerular spaces of the kidney. Evidences of smooth muscle spasm in the form of constricted pulmonary arterioles or bronchi were not a prominent or consistent finding. As the time interval between fever and death lengthened, the picture changed somewhat. Extensive hemorrhage in lungs, kidney, and spleen became the most prominent feature, and blood pigment was seen throughout these organs. Degenerative changes of both fatty and granular type were seen in the liver.

DISCUSSION

Kendell and Simpson[6] have shown that luetic patients sensitive to arsenical drugs can tolerate large doses of these chemicals during hyperpyrexia at temperatures 7.4 to 7.9° F. above the normal temperature (106.0 to 106.5° F.). Since the mean rectal temperature of a group of 57 of our normal guinea pigs at an environmental temperature of 65° F. was 102.8° F., the guinea pig temperature corresponding to the temperature used in treatment of human patients would be approximately 110° F. At this temperature we have obtained protection against acute anaphylactic deaths. While 110° F. in the guinea pig corresponds to the temperature at which human patients are treated, it did not appear that our animals could tolerate this temperature for any length of time because some of them were weak and listless. They did not, however, drag their hind feet as if paralyzed as described by Goldman,[3] although this was occasionally observed after the assaulting dose of horse serum had been given.

Deaths 1 to 24 hours following administration of serum in fevered guinea pigs have been encountered. These deaths did not occur when sensitized guinea pigs were fevered and injected only with saline. Furthermore, late deaths have not occurred in those animals used for trial runs to determine the best method for regulating the cabinet temperature to obtain satisfactory induction and maintenance of fever, nor in those animals which were fevered but for some reason were not injected with serum. Neither do late deaths occur very frequently when unfevered sensitized guinea pigs are injected intravenously with

horse serum, as shown by the protocols of the control animals given in Tables I, II, and III. Since these late deaths are not due to fever and do not occur very frequently in unfevered sensitized guinea pigs injected with serum, they are probably the result of anaphylactic shock superimposed on fever. It is suggested that these late deaths constitute another type of anaphylactic effect.

In our experiments three groups of animals of different ages were fevered at 108° F., and this temperature was maintained for 30 minutes before the assaulting dose of horse serum was administered. One group was seven weeks old, the other approximately 16 weeks (Table II, exp. 3), and the oldest group was 112 weeks of age. The 7-week-old animals obtained the least protection by this treatment; the 112-week-old animals, the most protection; while the 16-week-old animals were better protected than the youngest group of animals, but not as well protected as the oldest group.

SUMMARY

Sensitivity to horse serum in mature serum-sensitive guinea pigs is suppressed by artificially induced fever temperatures of 109 or 110° F. when the serum is injected as soon as these temperatures are attained.

Fever temperatures of 108° F. do not suppress anaphylactic shock if the animals are injected with serum as soon as they reach this temperature, but if this temperature is maintained for 30 minutes a certain degree of protection is afforded.

Serum-sensitive guinea pigs, 112 weeks old, are more readily protected than young guinea pigs, 7 weeks old, when subjected to a fever temperature of 108° F. for 30 minutes.

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THE CLUMPING OF ERYTHROCYTES IN HAYEM'S DILUTING FLUID: A GRAVE PROGNOSTIC SIGN*

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THE clumping of red cells, occasionally seen in Hayem's diluting fluid when performing an erythrocyte count, is a phenomenon which has been known for a number of years and has been observed in various pathologic conditions. This phenomenon is not uncommon and in our opinion has not received due consideration as a possible grave prognostic sign.

In his book, Gradwohl[1] reported, purely as a matter of record rather than as a diagnostic measure, three independent cases of clinical and pathologic Hodgkin's disease in which a peculiar condition of the red cells was noted. Distinct clumping of the cells occurred when the blood was diluted with Hayem's solution, making it difficult to perform an accurate erythrocyte count. The blood did not display any clumping in these cases, however, when diluted with a 2.5 per cent citrate of soda solution.

Kracke and Hoffman[2] in reporting a case state that "the erythrocyte count was impossible wth Hayem's diluting fluid because of marked clumping of the cells. A slight amount was present even when normal saline was used." Other similar reports are included with our observations in Table I.

Since no references in the literature were found attaching any prognostic importance to this manifestation, we considered it worth while to report some preliminary observations.

DESCRIPTION

In the clumping of the erythrocytes in Hayem's solution in the red blood cell pipette, the clumps, grossly, are small but vary in number and size, the larger ones sedimenting rapidly. (Fig. 1) They occur soon after dilution and appear to be permanent. Microscopically they appear as irregular entangled masses of red blood cells; some contain only a few cells, others a great many. As noted earlier, this definitely interferes with an accurate cell count, to a greater or lesser degree depending on the intensity of the reaction, which varies in different cases.

OCCURRENCE AND THEORY

The condition has been found to occur in a great number of entirely different diseases; however, we believe it to be based on the same fundamental blood change. In our study the cause was not determined, and although in some cases it might have been due to autoagglutination, in most instances it was probably an exaggeration of rouleau formation. This formation of irregular

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clumping sometimes simulates true agglutination, but is more properly called "pseudoagglutination." Belk[3] points out that "differences of opinion exist as to whether the pathologic cold agglutination and rouleau-forming substance are the same." He shows evidence that there is a difference. No doubt some cases of clumping of erythrocytes in Hayem's diluting fluid are brought about by autoagglutination, but most cases of autoagglutination have not shown this display.

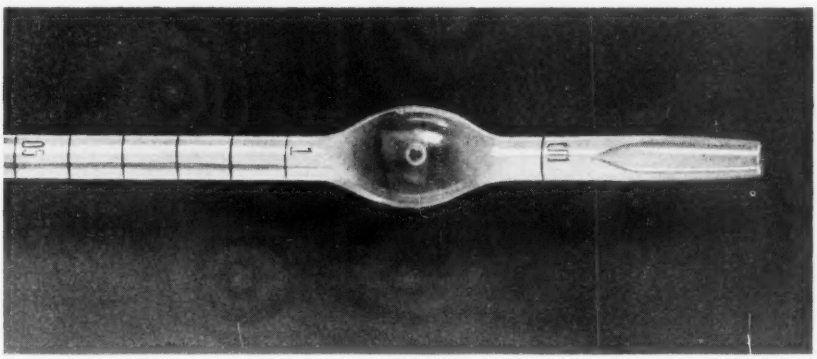

Fig. 1.

It has been found to occur in all ages of both sexes, and in various races (white, Negro, Indian.) Attempts to correlate this phenomenon with changes

TABLE I A

DATA SHOWING CLUMPING OF ERYTHROCYTES IN HAYEM'S SOLUTION IN INFECTIONS

DISEASE	OUTCOME		
	DEAD	LIVING	UNKNOWN
1. Pneumonia	8	2 (17 yrs. of age)	1
2. Bronchopneumonia	2		
3. Lung abscess	1		
4. Staphylococcus septicemia (osteomyelitis)	1		
5. *Streptococcus viridans* septicemia	2		
6. Subacute bacterial endocarditis	2		
7. Puerperal sepsis	2		
8. Abscess of kidney (Polycystic kidney and liver disease)	1		
9. Pyelonephritis—Bilateral (renal calculi)	2		
10. Pyonephrosis	1		
11. Pyelitis (also diabetes)	1		
12. Acute purulent otitis media (Staphylococcus septicemia)		2 (Age 4)	
13. Ulcerative colitis	1		
14. Upper respiratory infection (child)		1 (Age 4)	
15. Peritonitis (following appendicitis)	1		
16. Brain abscess			1
17. Cervical adenitis (child)	1		
18. Salpingitis			1
19. Meningitis (type?)	1		
20. Nephritis	1		
21. Rheumatoid arthritis (transfusions)	1		
22. Cholecystitis (transfusions)	1		
23. Chronic hemolytic anemia[1]	1		
24. Atypical pneumonia (acrocyanosis following)[4]		1	

in the blood protein, nonprotein nitrogen, sugar, white cell and differential counts, erythrocyte count, platelet count, blood groups, pathologic urine findings, fever, blood transfusion, etc., have failed. It may be associated with a high sedimentation rate and increased plasma protein, but it may also be seen with normal or even low protein levels.

TABLE I B

DATA SHOWING CLUMPING OF ERYTHROCYTES IN HAYEM'S SOLUTION IN NEOPLASMS AND ALLIED DISEASES

DISEASE	OUTCOME		
	DEAD	LIVING	UNKNOWN
1. Adenocarcinoma of stomach	1		
2. Scirrhous carcinoma of stomach	1		
3. Cancer of breast—carcinomatosis (1 had secondary thrombopenic purpura)	2		
4. Cancer of pancreas	1		
5. Cancer of kidney—carcinomatosis	1		
6. Cancer of throat	1		
7. Cancer ampulla of Vater	1		
8. Brain tumor (type?)	1		
9. Lymphatic leucemia (chronic) (1 had lobar pneumonia)	2		
10. Chronic leucemia (type?)[4]	1		
11. Myelogenous leucemia	1		
Myeloid leucopenic	1		
Leucopenic with secondary thrombopenic purpura	1		
12. Hodgkin's disease	3		
13. Multiple myeloma[5-9]	More than 5 (many)		

TABLE I C

DATA SHOWING CLUMPING OF ERYTHROCYTES IN HAYEM'S SOLUTION IN MIXED, METABOLIC, NONINFECTIOUS DISEASES

DISEASE	OUTCOME		
	DEAD	LIVING	UNKNOWN
1. Cardiac (chronic) failure	2		
2. Several transfusions (reason unknown)	1		
3. Burns		1 (Aged 16 years)	
4. Diabetes	1		
With cholecystitis and coronary occlusion	1		
Cardiorenal vascular disease	1		
5. Pernicious anemia	1		
6. Anaphylactoid purpura (terminal subacute glomerulonephritis, hepatitis, and bronchopneumonia)	1		
7. Essential hypertension	2		
8. Chronic nephritis (acute pericarditis, terminal)	1		
9. Raynaud's disease[10]		1	
10. Benzene poisoning (thrombopenic purpura)	1		

Tables I and II summarize the number and types of cases in which this reaction has been observed by us. In view of the number and variety of different diseases reported, it was not considered necessary to give complete case reports.

TABLE II

DATA OF TOTAL NUMBER OF CASES

		DIAGNOSED	UNDIAGNOSED
Total cases	105	72	33
Known dead	76	60	16
Known recovered	13	7	6
Outcome unknown	16	5	11

PROGNOSTIC SIGNIFICANCES

When this phenomenon occurs in a patient and is particularly intense, the usual outcome is death. Unfortunately it may make its appearance days, weeks, or even months before the patient's time of death. Although seen in all age groups, it is definitely more important and less variable in older age groups. The majority of cases in which the patient was known to have recovered were children. It is worthy of note that the reaction was reversible in cases where death did not occur. In general, slight or weak reactions were observed in cases that recovered, while strong reactions practically always indicated death as the final outcome.

SUMMARY

A phenomenon, namely the clumping of erythrocytes in Hayem's diluting fluid when performing a red cell count, has been described, and pathologic states in which it occurs have been tabulated. The possible importance of this phenomenon as a grave prognostic sign is offered.

ADDENDUM

A recent paper by Shone and Passmore[12] reveals that autohemagglutination is found almost constantly in atypical pneumonia, being more pronounced in the severe cases. This apparently minimizes the graveness of the phenomenon, but stimulates fresh interest for the study of the fundamental manifestation.

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REACTIONS IN THE BLOOD AND ORGANS OF DOGS ON INTRAVENOUS INJECTION OF A SOLUTION OF HEMOGLOBIN*

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THE presence of free hemoglobin in the blood plasma may be the result of poisonings (potassium chlorate, hydrogen arsenide, phenylhydrazine, naphthol, toluylene diamine, saponin, photodynamic fluorescent hydrocarbons and dyes [sulfonamides, acridine dyes]), blackwater fever, paroxysmal and cold hemoglobinemia, and resorption of large internal hemorrhages. In recent years hemoglobinemia has assumed an increased significance, because it may follow upon the transfusion of incompatible blood or of hemolyzed blood. Attempts have also been made to use solutions of hemoglobin freed from cellular stromata as a substitute for whole blood and plasma in the treatment of shock (Amberson;[1] Cannan;[2] O'Shaughnessy, Mansell, and Slome[3]). Such solutions can carry oxygen and maintain colloidal osmotic pressure for a period of up to 36 hours, after which they become ineffective because of the transformation of hemoglobin into methemoglobin and the escape of the injected hemoglobin from the blood with subsequent elimination through the kidneys (Lichty, Havill, and Whipple;[4] Klingmüller;[5] LaCoste, Aubertin, and Castagnou;[6] Yuile, Steinmann, Hahn, and Clark;[7] Gilligan, Altschule, and Katersky;[8] Hesse and Filatov[9]).

While it is a well-established fact that the intravascular hemolysis or the injection of hemolyzed blood may give rise to severe reactions, particularly anuria, caused by the blockage of the tubular lumina by hemoglobinous pigments and cellular detritus, such complications are less frequent or entirely lacking when solutions of pure hemoglobin are introduced (DeGowin, Osterhagen, and Andersch;[10] DeGowin, Warner, and Randall;[11] O'Shaughnessy, Mansell, and Slome[3]). In view of the practical and scientific significance of this aspect and as an additional contribution to the study on the effects of parenterally injected macromolecular substances (Hueper[12]), solutions of hemoglobin were injected into the veins of dogs.

EXPERIMENTAL PROCEDURE

A 2 per cent solution of hemoglobin (hemoglobin, pure, scales (Pfanstiehl)) in normal saline was injected into the jugular veins of three dogs (8.2 kg. to 10 kg.). One dog received 7 c.c. and two dogs 20 c.c. each of this solution. Blood studies were made 5, 15, 30, and 60 minutes, 2, 4, and 24 hours later to ascertain the acute hematic reactions. After an interval of three days, increasing amounts of hemoglobin solution were introduced daily five times per week and the blood was examined in weekly intervals. The individual dose was gradually

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raised to 70 c.c. within 5 weeks. The dogs were sacrificed by the intravenous injection of formalin solution after 6 weeks of treatment, during which 28 injections were given. A total of 1,390 c.c. of hemoglobin solution or 28 Gm. of hemoglobin were introduced into each dog during the experimental period.

The injections were usually well tolerated. Occasionally there occurred a short attack of dyspnea, rapid pulse, and, rarely, vomiting. After the first week of injections the urine showed a trace of albumin. During the six weeks of treatment the three dogs lost from 0.9 to 1.8 kg. of body weight.

ACUTE HEMATIC REACTIONS AFTER THE INTRAVENOUS INJECTION OF A SINGLE DOSE OF HEMOGLOBIN SOLUTION

Immediately after the intravenous injection of the hemoglobin solution the hemoglobin content of the blood was increased by 1 Gm. per cent to 1.8 Gm. per cent for a period of up to two hours. This increase was caused in part by the hemoglobin injected, as the plasma was distinctly hemolytic during the first 4 hours and slightly hemolyzed after 24 hours; in part it was the result of a transitory increase in the number of circulating erythrocytes (increases up to 1.8 million). The number of leucocytes, on the other hand, dropped sharply 5 to 15 minutes after the injection to values as low as 600 from an original level of 7,700 to 11,400. This leucopenia was accompanied by a relative lymphocytosis of moderate to marked degree. The original level was re-established after 30 minutes in the dog which received 7 c.c., but there was still a distinct lowering of the number of leucocytes in the other two dogs which were injected with 20 c.c. four hours after the injection. All three dogs revealed in the 24-hour test a considerable leucocytosis (22,000 to 33,200) associated with an increase of the neutrophilic cells and the appearance of an excessive number of immature leucocytes.

The volume of packed blood cells was slightly reduced at the 24-hour period, at which time there was also noted a moderately accelerated sedimentation of the erythrocytes (13 to 14 mm. Wintrobe-Landsberg hematocrit tubes). The coagulation time remained within normal limits in two dogs but showed 60 minutes after the injection an excessive lengthening (up to 2 hours) in the dog which had the most severe hemoclastic crisis. Normal values were present in all three dogs at the 24-hour period. The plasma viscosity, determined with the viscosimeter of Hess, remained within normal limits.

HEMATIC REACTIONS AFTER REPEATED INTRAVENOUS INJECTIONS OF HEMOGLOBIN SOLUTION

The repeated intravenous injection of hemoglobin solution caused a slow but progressive drop in the amount of hemoglobin, in the number of erythrocytes, and in the hematocrit values. The hemoglobin was reduced by approximately one-third of its original value toward the end of the 6-week period, while the number of erythrocytes had decreased by approximately the same value or more (30 to 50 per cent). The number of leucocytes fluctuated between 12,000 and 68,000 and was usually between 20,000 and 30,000. The sedimentation rate was considerably and consistently accelerated (30 to 55 mm.). The coagulation time and plasma viscosity remained within normal limits.

PATHOLOGY

The organs of the three dogs were grossly normal. Upon histologic examination the brain of one dog showed a moderately dense lymphocytic infiltration of the meninges and relatively dense perivascular lymphocytic accumulations around many of the intracerebral vessels. In some areas of the cortex there was a proliferation of capillaries and an increase of glia cells. The brains of the other two dogs were normal. The outer media of the aortas of two dogs showed in the region of the arch a crescent-shaped, large, hyaline, fibrohyaline, or mucohyaline area which contained an increased number of vasa vasorum with thickened walls and a moderate lymphoid cell infiltration. The inner media of the ascending aorta revealed a moderate to marked mucoid infiltration. The descending aorta was normal as were the larger aortic branches (carotid artery, femoral artery). The livers contained groups of Kupffer cells heavily loaded with a dark brown amorphous pigment. These storage phenomena were moderately frequent in the liver of one dog and scanty in those of the other two dogs. The liver cells of the three dogs had an almost unstained, coarsely flaky cytoplasm. The reticulum cells of the splenic pulp contained large amounts of brown pigment in one dog, scanty amounts in a second dog, and were almost free from pigment in the third dog. This animal, however, showed numerous megakaryocytes scattered throughout the pulp. The kidneys of two dogs were normal, while those of the third dog revealed moderate vacuolar changes in the tubular epithelium. None contained any brown pigment in the tubular epithelium or tubular lumina. The arteriolar walls in the testis of the one male dog were thickened and showed small, tongue-like endothelial processes. All other organs (hypophysis, thyroid, heart, lung, pancreas, stomach, intestine, adrenals, prostate, bladder) were normal. The bone marrow of the sternum consisted of a dense immature myeloid tissue.

COMMENT

The recorded observations show that the intravenous introduction of a relatively small amount of a comparatively dilute hemoglobin solution elicits a typical hemoclastic crisis, such as noted previously after the administration of other macromolecular substances (Hueper[12]). This hematic reaction is characterized by a primary transitory leucopenia with relative lymphocytosis, a secondary leucocytosis with absolute neutrophilia, increased sedimentation rate, and lengthened coagulation time. The moderate loss of body weight, the progressive and appreciable reduction in the number of erythrocytes and of the amount of hemoglobin, the considerable acceleration of the erythrocytic sedimentation, and the, at times, excessive leucocytosis represent manifestations following the repeated and prolonged intravenous administration of macromolecular material. It may be added that Gilligan, Altschule, and Katersky[5] under similar experimental conditions in man, and Yuile, Steinmann, Hahn, and Clark[7] found in dogs a lowering of the excretion threshold of the kidneys for hemoglobin.

It is remarkable that the histologic study of the various organs of the three dogs produced relatively little anatomic evidence pointing to a toxic action of the injected hemoglobin. It may be possible that the coarsely flaked appearance

of the liver cells indicates the presence of an extraneous proteinic matter interfering with the proper function of these cells. The hematogenous pigmentation of the Kupffer cells and of the splenic reticulum cells, on the other hand, are not sufficiently pronounced to cause any serious functional damage to these organs. The kidneys were practically without any significant lesions and showed a complete absence of any pigmentary deposits in the tubular epithelium and tubular lumina.

It is uncertain whether or not the meningo-encephalitis existing in one dog, the medial degenerations in the aorta of all three dogs, and the arteriolar thickenings in the testes of the one male dog have any causal connection with the treatment given or are coincidental findings. It may be mentioned in this connection, however, that Gilligan, Altschule, and Katersky[8] reported the occurrence of cramps in patients injected with hemoglobin solution and that Hesse and Filatov[9] observed marked spasms of the renal arterioles after the injection of hemoglobin solutions into animals, while von Berenesy[13] noted in a case of hemoglobinemia due to naphthol poisoning a hemoglobinous imbibition of the aortic wall.

CONCLUSIONS

1. The intravenous injection of hemoglobin dissolved in normal saline elicits a hemoclastic crisis followed by a leucocytosis within 24 hours.

2. Two of the three dogs used showed after the first injection an untoward reaction (dyspnea, rapid pulse, vomiting, bowel movement, urination, restlessness). None of the subsequent 27 injections was followed by such a response.

3. Repeated daily injections of hemoglobin solutions cause loss of weight, a considerable decrease in the number of erythrocytes and in the amount of hemoglobin, appreciable and, at times, excessive leucocytosis, and markedly accelerated erythrocytic sedimentation.

4. There was no formation of hemoglobinous casts in the tubular lumina and only a mild to moderate deposition of a hemoglobinous pigment in the liver and spleen.

5. The acute and chronic hematic and organic reactions follow in many respects the pattern set by observations made following the intravenous introduction of other macromolecular substances.

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CLINICAL CHEMISTRY

ASSAY OF RENIN SUBSTRATE BY LOW TEMPERATURE INCUBATION WITH RENIN*

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THE formation of angiotonin by the reaction between renin and renin substrate has been established to be enzymatic in nature (Plentl and Page[1]) (Leloir, et al.[13]). When tissue extracts are used as sources of renin and renin substrate, destruction of the angiotonin formed by this reaction occurs through the agency of the enzyme hypertensinase (Fasciolo, Leloir, and Munoz[2]) [angiotonase] (Helmer, Kohlstaedt, and Page [3]).

Renin and renin substrate are most conveniently assayed by determination of the angiotonin produced by their interaction. For the determination of renin, excess substrate is used, and the angiotonin produced is measured after constant incubation time (Leloir, Munoz, Braun-Menendez, and Fasciolo[4]). Renin substrate is determined by incubating it with excess renin and measuring the angiotonin produced after the reaction has gone to completion.[4]

In such methods, an accurate assay depends on the conversion of renin substrate to angiotonin *without consequent destruction of the angiotonin by hypertensinase*. This may be accomplished by destroying the hypertensinase present in tissue extracts. Two methods for inducing this destruction have been described (Dell'oro and Braun-Menendez;[5] and Braun-Menendez[6]), but unfortunately one of the methods results in partial destruction of renin substrate and is therefore useless when renin substrate determinations are desired, and the other has failed, in our hands, to destroy hypertensinase activity completely.

Page and Helmer[7] have demonstrated that the reaction between renin and renin substrate is decelerated but not stopped by decreasing the incubation temperature to 0° C. Furthermore, they observed no destruction of angiotonin in the time of their run (1½ hours). We have repeated and extended these observations, and have demonstrated that a decrease in temperature inhibits the destruction of angiotonin by hypertensinase far more than it does its formation by the renin-renin substrate reaction. At 0° C. the former reaction is entirely arrested, while the latter continues to completion, though at a diminished rate. We have made use of this phenomenon in the development of a technique for the quantitative assay of renin activator.

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MATERIALS

1. Renin:

Renin of sufficient purity for the present experiments and for ordinary pharmacologic investigations can be prepared in 2 to 12 hours (depending on the size of the batch) by the following technique:

Hog kidney (meat market kidneys are satisfactory) is ground through a meat grinder and agitated with five times its weight of 2 per cent NaCl. The mixture is run through a colloid mill, or blended with ice in a Waring blendor until homogenous. HCl is added with stirring to pH 4.1. The precipitate is removed by supercentrifugation or by filtration through Hyflo Supercel. An equal volume of saturated ammonium sulfate solution is added slowly to the filtrate, and the precipitate which is formed is filtered off on Hyflo Supercel. The filter cake is broken up, agitated with a volume of water equal to the original weight of kidney, and dialyzed in Visking tubing for 1 to 12 hours. The shorter times may be used if a rocking dialyzer and thin tubing are employed. The filtercel remaining in the solution is removed by filtration and the filtrate is saved. This solution, which is free of depressor activity and contains from 2 to 4 renin units (Swingle, Taylor, Collings, and Hays[8]) per c.c., is kept on ice or lyophiled. Lyophiled preparations contain approximately one gram of solids per pound of kidney.

This method has been applied to the preparation of renin from dog, rat, and hog kidneys with excellent results, but horse kidneys, for unknown reasons, fail to yield any pressor activity.

2. Renin substrate:

Renin substrate may be prepared from beef serum as described previously (Sapirstein, Southard, and Ogden[9]), or it can be assayed directly in serum or plasma.

3. Angiotonin:

A standard angiotonin for control of the pressor assays is prepared from renin and renin substrate by incubating 1 gram of lyophiled renin with 1 liter of renin substrate (equivalent to 5 liters of serum) for 2 hours at 0° C. The reaction is stopped by the addition of sufficient 50 per cent trichloroacetic acid to yield a final concentration of 5 per cent. The precipitate is removed by filtration, and the filtrate is boiled over a free flame for 30 minutes to remove the trichloroacetic acid (Peters and Van Slyke[10]). The solution is concentrated until 1 c.c. represents 10 c.c. of serum. One c.c. of this solution, which is stored on ice at pH 1.5 and neutralized immediately before use, contains 4 pressor units (Braun-Menendez, et al.[11]) and 0.03 mg. of non-ammonia N.

INCUBATION AND ASSAY METHODS

One gram of the standard lyophiled kidney powder is dissolved in 100 c.c. of 1 per cent NaCl. One c.c. of this solution is incubated with 10 c.c. of serum, plasma, or concentrated renin substrate for varying periods, and at different temperatures. At 0° the incubation is carried out for 0, 30, 60, 90, 120, and 1,000 minutes; at 25° for 7, 10, 15, 22, 30, and 45 minutes; and at 37° for 0, 3, 6, 9, 12, 15, and 18 minutes. The reaction is stopped by plunging the tubes into a boiling water bath and stirring violently while they are heating. Addition of

an equal volume of N/20 acetic acid during the heating facilitates the precipitation and results in a clearer filtrate. The precipitate is removed by filtration or centrifugation and the supernatant is assayed for pressor activity.

ESTIMATION OF PRESSOR ACTIVITY

Cats nembutalized with sodium pentobarbital are used. The blood pressure is recorded from the carotid artery. After administration of the usual anesthetic dose of sodium pentobarbital (35 mg. per kilo), the blood pressure is ordinarily found to be elevated and unstable and assays are inaccurate. This may be remedied by the slow administration of more nembutal via the femoral vein until the blood pressure drops to from 60 to 80 mm. Hg. At this level the blood pressure remains stable and the animal is quite responsive to angiotonin. Chloretone anesthesia, which produces a similar stable low pressure, has been found unsuitable for angiotonin assay, as the sensitivity is decreased threefold to tenfold.

The angiotonin content of the unknown solution is determined by comparison of its pressor activity with that of standard angiotonin. When it has not been possible to obtain identical responses from both standard and unknown, we have calculated the strength of the unknown angiotonin from the equation recommended by Braun-Menendez.[11]

$$\frac{(\text{Pressure rise unknown})^2}{(\text{Pressure rise standard})^2} = \frac{\text{Units of unknown}}{\text{Units of standard}}.$$ [11]

FINDINGS

The curves of Fig. 1*A* represent the angiotonin produced (plotted in units) at incubation temperatures of (a) 0°, (b) 25°, and (c) 37° C. In Fig. 1*B*, these three curves have been collated on an arbitrary, sliding time scale, all times being represented as the percentage of average value of the time required for maximum formation of angiotonin at each temperature.

TABLE I

AMMONIUM SULFATE FRACTIONATION OF RENIN SUBSTRATE IN BEEF SERUM

FRACTION	$(NH_4)_2SO_4$ CONCENTRATION	RENIN SUBSTRATE (UNITS/50 C.C. SERUM)
I	0-25	0
II	25-30	1
III	30-33	2
IV	33-36	8
V	36-40	5
VI	40-50	1

These results confirm those of Schales.[12]

DISCUSSION

It may be seen by inspection of Curve 1*B* that the ratio $\frac{\text{rate of destruction of angiotonin}}{\text{rate of formation of angiotonin}}$ decreases with temperature and that it assumes a value of 0 at 0° C.; i.e., hypertensinase activity is completely inhibited at this temperature. We have observed this elimination of hypertensinase activity in partially hemolyzed blood, and even upon incubating crude saline extracts of kidney with

whole blood. Since these contain large amounts of hypertensinase, it may be assumed that incubation at 0° C. completely inhibits the hypertensinase activity of tissue extracts.

The elimination of hypertensinase activity by 0° incubation, while renin activity remains, makes possible the easy, accurate assay of renin substrate by a procedure which prevents the destruction of formed angiotonin, and at the same time does not involve the partial destruction of renin substrate by measures designed to effect the chemical destruction of hypertensinase.

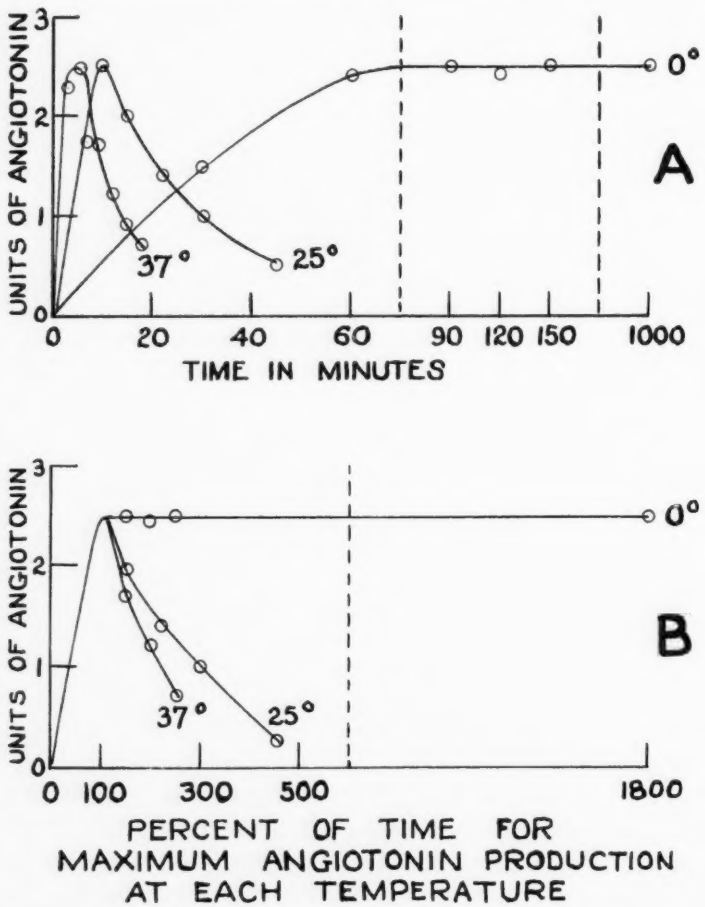

Fig. 1.—Effect of temperature on the formation of angiotonin by the action of renin on renin substrate.

A, Angiotonin production at 37°, 25°, and 0° C. plotted against time in minutes.

B, Angiotonin production at 37°, 25° and 0° C. plotted against time as per cent of time required for maximum production of angiotonin at each temperature.

APPLICATIONS

1. Assay methods: Renin substrate can be determined in whole blood, serum, plasma, or substrate concentrates without preliminary treatment by the following technique:

One part of lyophiled renin (see materials) is dissolved in 100 c.c. 1 per cent NaCl. One c.c. of this solution is incubated with 10 c.c. of the solution to

be assayed for two hours at 0° C. A control incubation of zero time is set up simultaneously. The reaction is stopped in a boiling water bath, according to the technique described above. Dilution with acetic acid is desirable in the case of whole plasma or serum but unnecessary when substrate concentrates are being assayed. The supernatant solution is assayed for pressor activity as previously described.

We define one unit of renin substrate as that amount of renin substrate which gives rise to one unit of angiotonin when the incubation is performed as described.

Using this assay technique, we have determined the substrate concentrations of various fractions of beef serum obtained by fractional precipitation with ammonium sulfate. Our results are expressed in Table I.

2. Preparation of angiotonin: Large batches of angiotonin can be prepared by the 0° incubation method as described above (materials). No precautions are necessary to avoid hemolysis, nor is the usual rigid time control, which is advisable in order to stop the reaction when maximum formation of angiotonin has occurred, necessary.

SUMMARY

1. A method for the quantitative assay of renin substrate based on the inhibition of hypertensinase activity at 0° C is described.

2. The bulk of the renin substrate of ox serum is precipitable between 0.33 and 0.40 saturation with ammonium sulfate.

3. Methods for the preparation of renin and angiotonin are described.

We wish to express our thanks to Mr. A. Robinson for preparation of the figure.

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LABORATORY METHODS

GENERAL

INDIRECT SPHYGMOMANOMETRY

A Physical and Physiologic Analysis and a New Procedure for the Estimation of Blood Pressure

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MANY sphygmomanometric devices and methods have been devised for indirect blood pressure estimation. All procedures thus far described in the literature cannot be considered totally free from error under clinical conditions. The object of this investigation, therefore, is to evaluate the sources of error in the indirect sphygmomanometric methods and to suggest procedures for eliminating or reducing the magnitude of these errors.

To proceed with any degree of success, we found it necessary to devise an apparatus which could register graphically and simultaneously all of the physiologic phenomena which must be evaluated when estimating blood pressure by the palpatory, oscillatory, and auscultatory methods. Such a device, obviously, was intended as a means for the elimination of the human element with its subjective differences in the appreciation of the physiologic signs and to make possible a quantitative comparison of the associated phenomena.

REVIEW OF LITERATURE

In 1733, Stephen Hales[1] performed an experiment in which he estimated the blood pressure in a horse. Hales inserted a cannula into the femoral artery and allowed the blood to rise in a vertical tube; from the height of the blood column, Hales calculated the pressure. In 1828, Poiseuille[2] substituted a mercury manometer for the Hales' tube, and in 1847 Ludwig[3] devised a direct recording manometer. Faivre,[4] Albert,[5] Müller and Blauel,[6] Dehan, Dubus, and Heitz,[7] continued the work.

In recent years, many refinements have been added to the Hales' apparatus. Some of the better known investigators who have made worth-while contributions to the art are Galatà,[8] Poillet, Dodel and Boucomont,[9] van Bogaert, Beerens, Lequime and Samain,[10] Giroux,[12] Dameshek and Loman,[13] Hamilton, Brewer, and Brotman,[14] and Macleod and Cohn.[15]

It is generally agreed that the direct communication method between artery and registration device is the most accurate means for measuring blood pressure. Possible errors in the direct method due to nonabsolute transmission of pres-

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sure from artery to instrument, spastic contraction of the artery in which the cannula has been introduced, psychic factors, the difficulty at times of accurately ascertaining the exact zero pressure level, and a few other such factors may exist. Although the direct method is invaluable in the physiologic laboratory, the procedure of inserting a cannula into the artery of a patient is a decided limitation from a clinical standpoint.

Riva-Rocci[16] in 1896 introduced an indirect method for blood pressure estimation based upon the preliminary findings of Béhier,[17] Foster,[18] Landois,[19] Philadelphien,[20] von Basch,[21] and Potain.[22] The Riva-Rocci method utilized a controlled pneumatic cuff for compressing the arm (brachial artery) and the palpation of the radial artery. The first palpable pulse wave which appeared during a gradual decompression of the cuff was taken as a sign that the level of systolic pressure had been reached.

The original palpatory method of Riva-Rocci is in use today as a check on the auscultatory systolic pressure. The accuracy of the method is limited by the sensitivity of the fingers in detecting the first small waves of the pulse, which may result in an underestimation of the systolic pressure. The estimation of the diastolic pressure by the Riva-Rocci method is an extremely uncertain procedure.

In order to improve upon the Riva-Rocci palpatory method, Bing,[23] Pal,[24] Vaquez,[25] and others devised visual oscillatory indicators. These devices utilized a second cuff as a substitute for the fingers, which cuff transmitted the arterial pulsations into a device which produced a magnifying effect with resulting easier perception than was possible by palpation. Some years later a double cuff was used by Groedel and Miller[26] for the registration of the pulse.

The Riva-Rocci palpatory method and the oscillatory indicators, plus the investigations of Marey[27] and von Recklinghausen,[28] paved the way for the first practical oscillatory type sphygmomanometer by Pachon.[29] The Pachon apparatus immediately received wide usage and is in use to this day. It is composed of an aneroid manometer which indicates the cuff pressure and of a constantly balanced manometric capsule which indicates the magnitude of the arterial oscillations.

In using the instrument, the cuff is applied to the arm in the conventional manner, and its pressure is raised to a point above systolic. The cuff pressure is indicated on the aneroid manometer. Attention is then focused upon the indicator controlled by the balanced manometric capsule, and it is observed for oscillations. This procedure is repeated as the cuff pressure is brought down to below diastolic pressure, usually in steps of 5 to 10 mm. of mercury.

As the cuff pressure is gradually lowered (in steps) from above systolic, a series of oscillations of small amplitude appear (supramaximal oscillations). This series of oscillations has been attributed to the hydraulic ram action of the pulsating proximal stump of the artery against the edge of the cuff. The supramaximal oscillations are then followed by a marked and abrupt increase in the oscillatory amplitude (the level of systolic pressure); the oscillations continue to increase progressively in amplitude to a maximum, then gradually decrease, and then abruptly decrease followed by some small oscillations (inframinimal oscillations).

The diastolic pressure was first thought to coincide with the maximal oscillation, as the arterial wall should then oscillate freely between two equivalent pressures (Marey[27]). As a result of the investigations of Macwilliam, and Melvin,[30] whose data were accepted by Gallavardin,[31] the diastolic level was placed much lower (where a marked change in amplitude occurs). Pachon and Fabre,[32] and Gley and Gomez[33] also confirmed the necessity of the change and accepted the data.

Gallavardin[34] later modified the Pachon oscillometer and the cuff, and concluded that the supramaximal oscillations must be due to the hydraulic ram action. Fabre[35] attributed the supramaximal oscillations to the elastic reaction of the artery; Gley and Gomez[33] attributed the phenomenon to the infundibulum formed by the upper part of the artery, and Minerbi and Merighi[36] to the pulsations of the arteria brachialis profunda. For more precise oscillometric investigations, recording oscillometers were devised by Plesch[37] (tonoscillograph) and Boulitte[38] (arterial oscillograph).

The investigations of Strasburger[39] and Ehret[40] were continued by Korotkow.[41, 42] Korotkow, in 1905, suggested that blood pressure may be estimated by auscultation. The Korotkow method utilizes a Riva-Rocci apparatus and a stethoscope. Instead of palpating the artery, as is done by the Riva-Rocci palpatory method, a stethoscope is applied to the brachial artery below the cuff. Five distinct phases may be heard and differentiated, as the cuff pressure is slowly decreased from above systolic pressure to below diastolic. The five phases occur in the following order: Sounds similar to the first cardiac sound; a similar series of sounds, plus a hissing murmur; the vanishing of the murmur with the sound remaining; a sudden muffling of the sound; the disappearance of the sound.

Since the introduction of the Korotkow auscultatory method, many differences of opinion have been expressed as to the true location of the diastolic pressure. At first, the level where the loudest sound occurred was considered the diastolic level. Later, the idea prevailed that the last sound should be taken as the diastolic level (Macwilliam and Melvin;[30] Erlanger[43]). At the present time, the general belief, as expressed by the Anglo-American Committee,[44] is that the level of systolic pressure is the beginning of the first sound phase or where the first sound regularly appears; the diastolic pressure where the sounds suddenly become dull and muffled. If there is a difference between that point and the level at which the sounds completely disappear, the American Committee recommends that the latter reading should be regarded also as the diastolic pressure. The British Committee believes that except in aortic regurgitation it is nearly always possible to decide the point at which the abrupt change comes, and that this is the only reading that should be recorded.

Indirect methods for bloood pressure estimation, as previously mentioned, are subject to several possible sources of error. The instrument itself may introduce variable degrees of error, but a more serious source of error is the utilization of the human senses for quantitative measurement. Cook,[45] and more recently, Wright, Schneider, and Ungerleider[46] have demonstrated that marked discrepancies occur in the taking and interpretation of blood pressure by competent physicians.

Since Marey's investigations in 1881, subjective differences in the evaluation of the blood pressure have been appreciated. As a result graphic registration methods have been devised by Béhier,[17] Foster,[18] Landois,[19] Philadelphien,[20] Jaquet,[47] Gibson,[48] Erlanger,[49] Uskoff,[50] Silbermann,[51] Brugsch,[52] Münzer,[53] Fleischer,[54] and others. All of these devices operated on the spygmographic principle. The instruments of Boulitte[38] and Plesch,[37] which have been mentioned, should also be included in this category.

More recently, Gomez and Langevin[55] utilized a piezo-electric quartz crystal and electronic amplifier to register the pulse. The characteristic advantages inherent in the piezo-electric crystal[56, 57] for this type of application were lost, because the pulse was transmitted to the crystal through a contact button, thus reverting back to the Potain method. This is, the arterial pulsation is registered through the uncertain transmission of a contacting button with the result that all the errors present in the old Potain method are still present and possibly even magnified.

Von Recklinghausen[58] observed that graphic registrations of the pulse during a deflation of the cuff from above systolic pressure to below diastolic show small changes in the shape of the pulse waves which indicate the lateral and end systolic pressure, and the lateral and end diastolic pressure. Bazett and Laplace[11] confirmed von Recklinghausen's observations.

Von Recklinghausen[28] in 1901 suggested that the width of the cuff may severely affect the accuracy of the blood pressure reading. He showed that a cuff which is too narrow gave too high a blood pressure reading. The correctness of von Recklinghausen's observation was confirmed by Gumprecht's[59] experiments on the cadaver with artificial circulation and by Müller and Blauel,[6] Janeway,[60] and Ragan and Bordley[61] on living human subjects. Ragan and Bordley show experimentally that "in some obese subjects the substitution of a wide (20 cm.) blood pressure cuff for the standard (13 cm.) cuff afforded more accurate auscultatory measurements of both systolic and diastolic pressure. In most subjects, however, the wide cuff yielded measurements which were too low."

DESCRIPTION OF APPARATUS

In Fig. 1*A* may be seen a schematic diagram of an apparatus which is capable of registering simultaneously all of the physiologic phenomena that must be evaluated when estimating blood pressure by the auscultatory, palpatory, and oscillatory methods.

The apparatus consists of a double chamber pneumatic cuff; the outer covering of the twin chambers is of the usual nondistensible material. The upper or pressure chamber is of the usual dimensions (13 × 25 cm.); the lower or registration chamber dimensions are 3 × 25 cm. The pressure and registration chamber dimensions may be varied to suit any particular application or may be placed in separate nondistensible bags; a single bag, however, simplifies the application procedure.

The pressure chamber of the cuff connects to a manometer of the recording or visual types or both as well as to a needle valve and inflation pump. The combination of pressure chamber, needle valve, and inflation pump may com-

municate with or be cut off from the rest of the pneumatic system by the setting of valve "A."

The piezo-electric transducers are essentially differential rochelle salt crystal microphones. A rochelle salt crystal microphone is normally incapable of withstanding any appreciable pressure without probable damage to the crystal. In an application such as this, it is essential to employ some pressure in the registration chamber. As a result, the registration chamber pressure is allowed to communicate with the front and rear of the diaphragms in the piezo-electric transducers when valve "B" is in the "open" position. When the front and rear pressures are identical, the diaphragms are not distended and the crystals in turn are not flexed. Should valve "B" close, the trapped pressure to the rear of the diaphragms will be maintained identical with the pressure in the registration chamber. Any arterial stimulations that are now applied to the registration chamber of the cuff will distend the diaphragms and flex the crystals in proportion to the differential pressure.

A detailed technical discussion of the design and mathematical theory of the differential piezo-electric crystal transducers suitable for the registration of the Korotkow sounds and the arterial pulsations in sphygmomanometry is obviously beyond the scope of this paper. However, a general consideration of some of the more important features should be of interest.

The heart of the differential piezo-electric transducer is a cartridge or diaphragm type of crystal unit similar to that described by Rappaport and Sprague.[56] "It consists of a diaphragm which is directly coupled to a bimorph rochelle salt crystal through a tiny connecting rod; the crystal and connecting rod are located in the interior of the cartridge. Should an actuating force be applied to the diaphragm, proportional stresses will be set up in the crystal, and it will transform these stresses into equivalent electrical potentials which are conducted away from the crystal by means of metallic foil electrodes in contact with it."

According to Rappaport and Sprague, "the fundamental frequency, or natural period, of the bimorph crystal is approximately 10,000 cycles per second. When a diaphragm is coupled to the crystal, the fundamental frequency of the combination is lowered to a few thousand cycles per second." This is well above the upper frequency limits which are present in the Korotkow sounds and in the arterial pulsations.

The attenuation effects throughout the registration frequency spectrum are governed by the electrical, electronic, and galvanometric systems. The channel which registers the Korotkow sounds is identical with that described by Rappaport and Sprague wherein the over-all frequency response of the system is similar to that of the "average acoustic stethoscope." The over-all frequency response of the arterial pulsation registering channel is identical to the sphygmographic arrangement described by Rappaport and Sprague which is flat from practically zero cycles per second to the upper limits of the galvanometric system. An electrocardiographic channel (electronic type) is used for the registration of the arterial pulsations. An electronic type electrocardiographic channel normally possesses a speed of response of 0.01 second or faster.

The method of Miller and White[57] is employed for making possible the utilization of a piezo-electric crystal for the registration of frequency components which approach zero cycles per second, such as are encountered in arterial pulsations.

The essential difference between the piezo-electric transducers employed in this sphygmomanometric apparatus and the sphygmographic systems described by Miller and White and Rappaport and Sprague is the differential arrangement which permits operation at pressures above or below atmospheric.

In Figure 1*B* is shown the sphygmomanometric apparatus used in this investigation connected to an electronic type electrocardiograph—phonocardiograph.

OPERATING TECHNIQUE

The double chamber cuff is applied to the subject as is done with the usual type of sphygmomanometer (Fig. 1*A*). With tandem valves "A" and "B" open, and the needle valve closed, the system is inflated by means of the pump. When the valves are in this position, all the component pneumatic channels are in direct communication and under identical pressure; the pressure is indicated

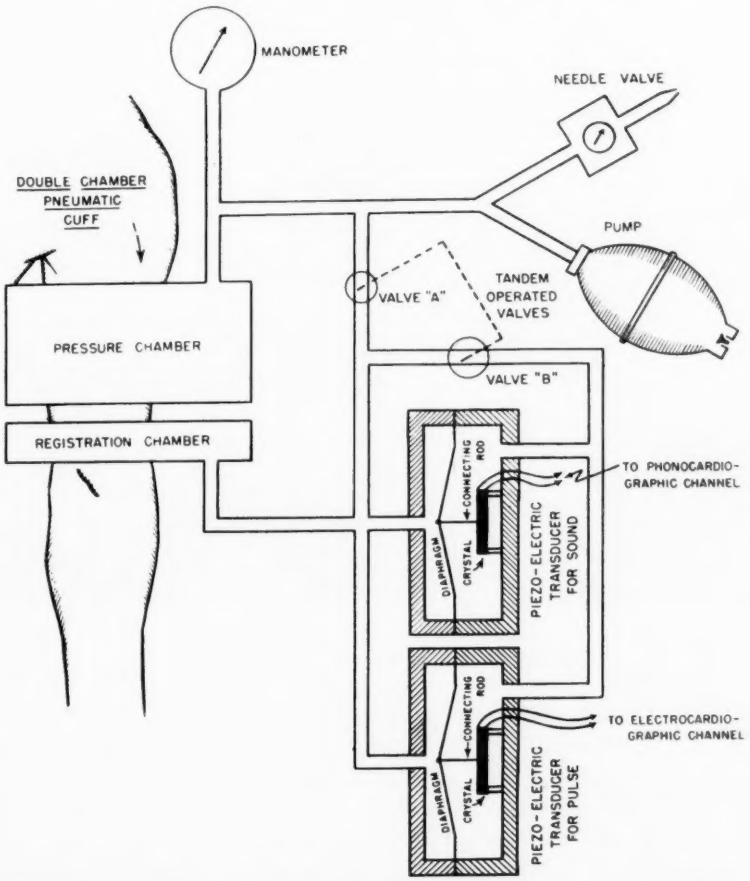

Fig. 1*A*.—Schematic diagram of an apparatus which is capable of registering simultaneously all of the physiologic phenomena that must be evaluated when estimating blood pressure by the auscultatory, palpatory, and oscillatory methods.

by the manometer. When a pressure of about 20 mm. of Hg is attained, tandem valves "A" and "B" are closed. The closure of valve "A" segregates the pressure chamber of the cuff, the manometer, the needle valve, and pump from the registration chamber of the cuff, valve "B," and the piezo-electric transducers. The closure of valve "B" traps a noncommunicable pressure or cushion of air at the rear of the diaphragms in the piezo-electric transducers equal to the pressure in the registration cuff and the front of the diaphragms.

The pressure is now increased in the pressure chamber of the cuff by means of the pump to above the anticipated systolic pressure. By means of the needle valve, the pressure chamber is gradually deflated. During the deflation period, the registration chamber of the cuff detects the Korotkow sounds and pulse when present and transmits both to the piezo-electric transducers.

Fig. 1*B*.—Sphygmomanometric apparatus used in this investigation connected to an electronic type electrocardiograph-phonocardiograph.

The piezo-electric transducer for sound and its associated phonocardiographic system is sensitive to the vibratory frequencies present in the Korotkow sounds but not the low-period vibrations present in the pulse. On the other hand, the piezo-electric transducer for pulse and its associated electrocardiographic system is sensitive to the low-period vibrations present in the pulse. The result is a simultaneous registration of the Korotkow sounds, the pulse, and the cuff pressure (pressure chamber) during the deflation period. It should be mentioned at this point that the phonocardiographic registration system records

the Korotkow sounds with the same degree of modification[16] as produced by the average acoustic stethoscope; logarithmic registration[62, 63] may be used as well.*

THEORY AND OBSERVATIONS

Wiggers[64] in his description of the central arterial pulse and its conversion into the peripheral arterial pulse states: "The central pulse, as the pulse in the large arteries near the heart is called, shows (Fig. 2) first two preliminary vibrations: One due to auricular systole, *a-b*, the other to the isometric rise of tension in the ventricle, *b-c*. These are followed by a sharp primary oscillation, *c, d, e*, 0.013 to 0.02 second in duration and beginning at the ejection of blood

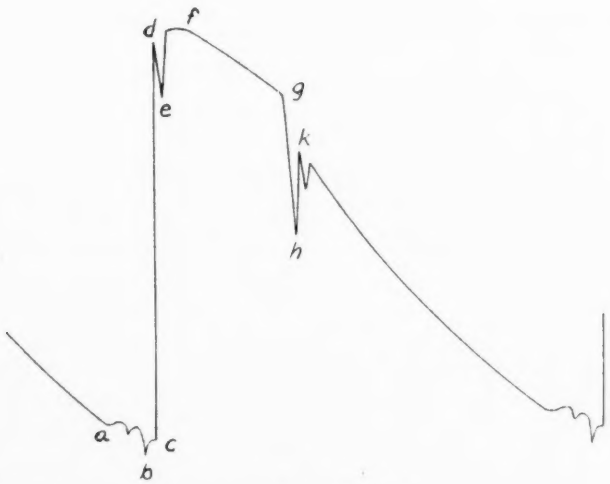

Fig. 2.—The central arterial pulse (after Wiggers).
a, b, Vibrations due to auricular systole.
b, c, Vibration due to the isometric rise of tension in ventricle.
c, d, e, Oscillation due to sudden ejection of blood.
e, f, Pressure rise when ventricles and arteries form a common cavity.
f, g, Gradual pressure fall during rest of systole.
g, h, Sudden drop of pressure at the beginning of the ventricular relaxation (diastole).
k, After vibrations of valves and blood column.

into the aorta. It is due to the fact that the sudden ejection has set the arterial column in vibration. After this vibration the arterial curve follows the intraventricular pressure, for now ventricles and arteries are a common cavity. First, the pressure rises, *e, f*, thereafter reaches a more or less definite summit, and then falls gradually during the rest of systole, *f, g*. At the beginning of the ventricular relaxation (diastole), there is a rapid backward movement of the blood toward the heart, causing the pressure to fall suddenly, thus creating the incisura of the central pulse, *g, h*. Several after-vibrations of the valves and blood column, *k*, recognized over the chest as heart sounds, follow. The pressure then falls smoothly except for a few oscillations due, no doubt, to reflections from the peripheral bifurcations of arteries [Frank[65]]."

*The stethoscopic system of phonocardiographic registration does not register the sound vibrations as they exist at the source, but as they are presented to the ears of an observer by an average acoustic stethoscope. Logarithmic phonocardiography is a graphic representation of sound vibrations as they are perceived by the average observer of normal hearing when an average acoustic stethoscope is employed. Logarithmic phonocardiography is thus the summation of the stethoscopic and human hearing modifications.

"These complicated series of pressure variations present in the aorta and large arteries are modified in their peripheral transmission by friction and interference with reflected waves (Frank). Or, one may state the case differently by saying that the vascular system represents a manometer system, the ability of which to transmit the pressure variations in the central arteries faithfully to the periphery becomes less and less as the length of the column increases [Weber[66]]."

"The changes actually noted as we pass, step by step, to the peripheral vessels are: The preliminary and primary oscillations are damped and obliterated; the sharpness of the incisura is reduced and finally replaced by a rounded dicrotic dip and elevation; the upstroke is delayed and becomes more gradual; the tops are more rounded and the amplitude smaller until in the smallest arteries and capillaries the pulse is entirely obliterated."

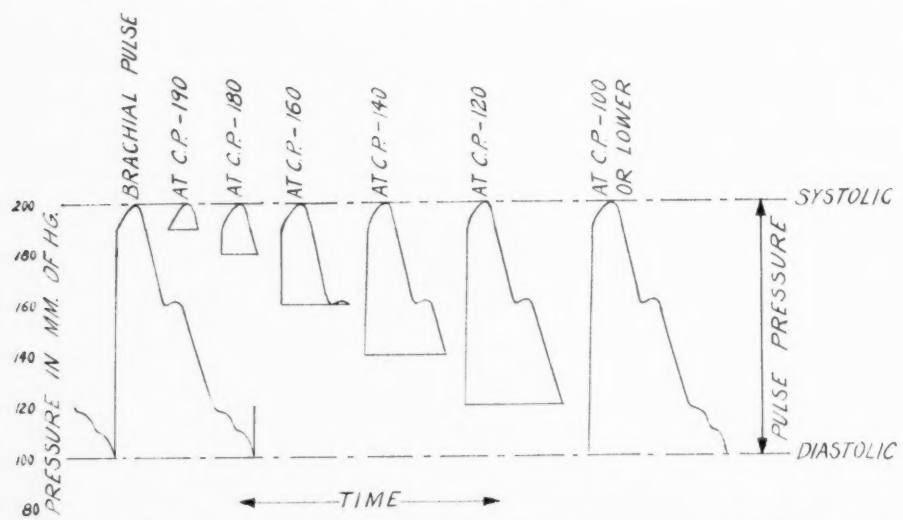

Fig. 3.—A graphic representation of the degree of modification various cuff pressures (above diastolic) produce upon the brachial pulse.

Wiggers further states: "Frank, however, finds difficulty in interpreting the dicrotic wave as merely the incisura and after-vibration modified in transmission, for the dicrotic wave becomes larger toward the periphery and all other waves submitted to the same frictional influence become smaller. He, therefore, believes that its amplitude is augmented by a resonance effect with other vibrations reflected from the periphery."

When a pneumatic blood pressure cuff is applied over the brachial artery, inflated to a pressure above systolic, and allowed to deflate gradually, as is done when estimating the blood pressure, the pulsation in the brachial artery immediately below the cuff is modified. Fig. 3 is a graphic representation of the degree of modification at various cuff pressures. The pulsation marked "brachial pulse" is the usual configuration of the pulse in the brachial artery in which many of the finer oscillations so evident in the central pulse are completely attenuated.

Fig. 3 indicates graphically that when the cuff pressure is above the systolic level, 200 mm. of Hg in this case, the brachial artery is collapsed and no pulsa-

tion is detectable immediately below the cuff. With a cuff pressure slightly less than the systolic level (190 mm. of Hg), a slight pulsation is present in the brachial artery just below the cuff. This pulsation represents the uppermost portion of the brachial pulse, the pressure level of which exceeds the cuff pressure and allows the collapsed brachial artery to open slightly during this short interval. As the cuff pressure is lowered, more of the brachial pulse is passed through the artery because the artery below the cuff is in a collapsed condition a shorter period of time. When the cuff pressure approaches the diastolic level or below, only then is the brachial pulse completely transmitted and at no time during the cardiac cycle is the brachial artery collapsed.

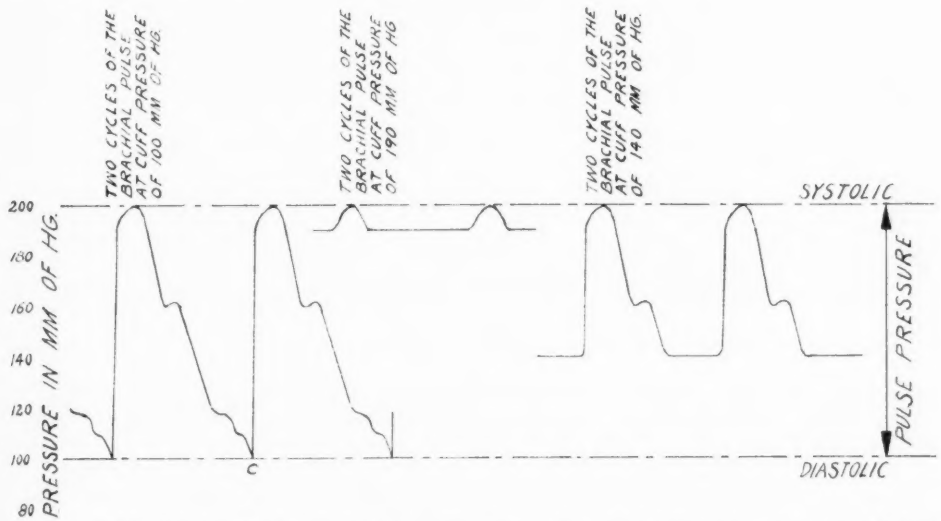

Fig. 4.—Theoretical configuration of two succeeding cycles of the brachial pulse at different values of cuff pressure (above diastolic).

Thus, the systolic pressure in the brachial artery may be estimated by the introduction of a sphygmographic device which is capable of detecting the pulsation of the artery just below the pressure cuff. That is, the systolic level is the point at which the first trace of brachial pulsation is detectable as the cuff pressure is gradually lowered from above the systolic level. This corresponds to the pressure level where the uppermost portion or crest of the brachial pulse (Fig. 3) is greater than the cuff pressure and the artery opens slightly during that instant. In Fig. 4 are shown two succeeding cycles of the brachial pulse (blood pressure 200/100), two cycles at a cuff pressure of 190 mm. of Hg, and two cycles at 140 mm. of Hg. Note the theoretical configuration of the sphygmogram when the systolic pressure is more than the cuff pressure. Also note the manner in which the lower pressure components of the pulse are obliterated by a flattening process due to the collapse of the artery when the cuff pressure is more than the diastolic level. This sphygmographic characteristic permits the estimation of the diastolic level[11] in the following manner: As the cuff pressure is gradually dropping from above the systolic level, the recorded brachial artery pulsations become larger and larger, but a rather flattened base line is present between adjacent cycles until the diastolic level

is reached where the flattening effect due to artery collapse vanishes. The cuff pressure at which the first brachial pulse appears with a well-defined and peaked contour (as represented by *c* in Fig. 4) is the diastolic level.

Let us now analyze an actual brachial pulse tracing as the cuff pressure is gradually lowered from above the systolic level to well below the diastolic level (Fig. 5). The graphic record was obtained with the apparatus show in Figs. 1*A* and 1*B*. The registration cuff was inflated to a pressure of 20 mm. of Hg, and the pressure cuff was varied as indicated on the record.

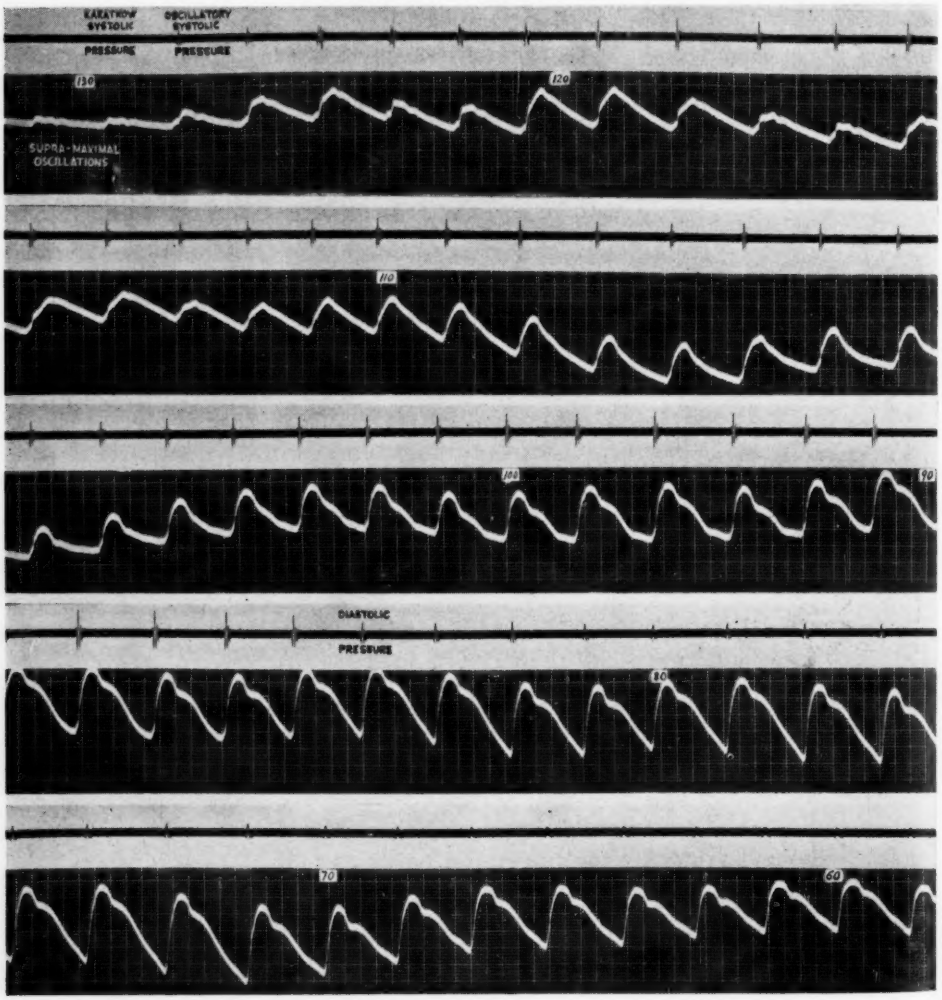

Fig. 5.—A simultaneous registration of the Korotkow sounds and the brachial pulsations (oscillatory curve) during a cuff deflation from above systolic blood pressure to well below the diastolic pressure level. The pressure in the pressure cuff is indicated by the figures in the white squares; the registration cuff was kept at a constant pressure at 20 mm. of Hg. Stethoscopic registration was employed in the sound track.

Fig. 5 shows the typical supramaximal oscillations* of small amplitude which are followed by a series of gradually increasing pulsations. The first

*The supramaximal oscillations are not due to passage of blood beneath the cuff but are produced by the "ram action" of the preliminary vibrations $a - b$ and $b - c$ of Fig. 2 against the cuff. Mechanical vibrations which are detected by the registration cuff are thus set up in the arm. The supramaximal oscillations definitely precede the sharp primary oscillation which is clearly shown in Fig. 5.

distinct pulsation after the supramaximal oscillations is obviously the level of systolic pressure. As indicated in Figs. 3 and 4, a gradual increase in the amplitude of the brachial pulse is expected as the cuff pressure is gradually lowered. The pulse amplitude seems to reach a maximum amplitude between approximately 90 and 70 mm. of Hg cuff pressure and thereafter attains a constant amplitude of pulsation. This is exactly what one would expect to see in an oscillometric curve from a theoretical viewpoint, except for the increase in amplitude between approximately 90 and 70 mm. of Hg. As previously mentioned, Marey[27] attributed this phenomenon to a resonance effect where the arterial wall oscillates freely between two equivalent pressures or what he believed was the diastolic level. Such a resonance effect obviously does take place and is clearly shown in practically all the graphic records we have obtained. This resonance effect, however, is too broad to be useful for accurately evaluating the level of the diastolic pressure. The "flattening effect" just above diastolic pressure and the distinct appearance of a "negative dip" (observed by von Recklinghausen[58] and Bazett and Laplace[11]) just below the diastolic level is more accurate. Note how easy it is to locate this point in Fig. 5, wherein it falls roughly halfway in the resonance region. Theoretically, the termination of the flattening effect should occur exactly in the center of the resonance region.

In Fig. 5, a very faint Korotkow sound appears somewhat earlier than does the first distinct brachial pulsation. Thereafter, a Korotkow sound appears with every brachial pulsation. After the faint first Korotkow sound, the Korotkow sounds gradually increase in intensity for a period of time as the cuff pressure is gradually lowered.

The Korotkow sounds occur simultaneously with the sharp primary oscillation of the brachial pulse or during the beginning of the ejection of blood into the artery. During the rapid ejection interval, the blood velocity is greatly increased when passing through the partially collapsed or constricted portion of the artery immediately below the pressure cuff. This rapid blood velocity in turn sets the artery and surrounding tissue into vibration with the resulting sound. Another contributing factor to the production of the Korotkow sound is the rapid distention of the artery and surrounding tissue by the sharp primary pulse oscillation. A rapid displacement of tissue in a relatively confined area bounded by the nondistensible cuff will produce audible sound waves.

As the cuff pressure is gradually lowered from the systolic level, the arterial pulsations and the Korotkow sounds gradually increase in intensity. A broad resonance effect as previously described occurs in the arterial pulsation in the region of the level of diastolic pressure and thereafter maintains a more or less constant amplitude. The Korotkow sounds, however, suddenly become diminished in intensity at the diastolic level and thereafter become less and less intense. This Korotkow sound intensity effect at the diastolic pressure level may be explained by the two primary causes for its production. That is, when the cuff pressure is less than the diastolic level, there is no longer any arterial constriction with abnormally increased blood flow velocity immediately below the cuff. Also, the rapid distension of the artery and surrounding tissue by the sharp primary oscillation takes place in a relatively less and less confined area as the cuff pressure is gradually lowered. In other words, the Korotkow sound

which is produced when the cuff pressure is just above the diastolic level has both sound generating factors operating at maximum efficiency. But, when the cuff pressure is just below the diastolic level, the former becomes minimal and the latter gradually reduces with the gradual lowering of the cuff pressure.

It is of interest to note that in the blood pressure record of Fig. 5, the first Korotkow sound precedes the first distinct arterial pulsation by approximately 2 mm. of Hg. The abrupt dropoff in the intensity of the Korotkow sound which indicates the diastolic blood pressure level corresponds exactly with the termination of the negative flattening effect of the brachial pulse. In some persons, the first distinct Korotkow sound may precede the first distinct arterial pulsation by as much as 5 mm. of Hg, and vice versa; the discrepancy at diastolic level rarely exceeds 5 mm. of Hg.

Another sound that is commonly registered in the blood pressure graph occurs simultaneously with the incisura of the arterial pulse, the notch preceding the dicrotic wave. The mechanism of the production of this sound is identical to that of the Korotkow sound. Its intensity is much less than the Korotkow sound because the energy generated by the sudden drop in pressure is obviously of lesser magnitude than that due to the rapid ejection of blood into the aorta. The secondary sound which occurs simultaneously with the incisura may be seen in most of the cycles of Fig. 5 but is most distinct below the diastolic level.

Considerable error may be introduced when estimating the systolic blood pressure by the palpatory method. The degree of error may be in the order of several millimeters of mercury below the actual value because the primary oscillation which is palpated is so slight in intensity that it falls below the threshold of human feeling. The diastolic level definitely cannot be determined by palpation, because the sense of human feeling cannot differentiate the point of transition where the negative flattening effect becomes peaked. To depend upon the resonance effect for diastolic blood pressure evaluation is obviously uncertain by the palpatory method because the sense of human feeling is insufficiently sensitive for the accurate detection of the apex of resonance; even graphic registration cannot be depended upon because of the broad resonance effect. Furthermore, it is doubtful whether this resonance effect is palpable in most human beings.

The oscillatory method is reliable in the estimation of the systolic blood pressure only when accurate sphygmographic registrations are made, wherein the phase corresponding to the ejection of blood into the aorta is easily differentiated from the supramaximal oscillations. The sharp primary oscillation, when it first appears at systolic level, is very difficult to differentiate from the supramaximal oscillation by means of a visual type indicator. Usually, the first noticeable sharp primary oscillation via a visual indicator is several mm. of Hg below the actual systolic level. The graphic method requires a paper or film speed of not less than approximately 25 millimeters per second. Slower speeds bunch the component pulse waves too close together making it rather difficult to determine the initial occurrence of the sharp primary oscillation. In most persons the diastolic pressure may be estimated by the negative flattening effect; this phenomenon is not distinct in a small percentage of human beings when using the graphic method and rarely observable via the visual method.

Most all recorded oscillometric curves that are described in the literature show a gradual tapering off of the pulse amplitude as the cuff pressure is lowered gradually below the diastolic level. Our registrations do not show this tapering-off characteristic. *The reason the pulse tapering-off phase is registered by most oscillometers is not because the pulse vanishes, but because the efficiency of the cuff in detecting the arterial pulsations diminishes as the cuff pressure is lowered.* Therefore, it is incorrect to use the pressure cuff as a detector of arterial pulsations as well as a means for collapsing the artery. The use of independent cuffs, one as a pressure cuff for collapsing the artery and the other for detecting the pulsations, is the more accurate method. The latter cuff, however, must be kept at a pressure well below the diastolic level. We have found a pressure of approximately 20 mm. of Hg generally satisfactory. Other well-known sphygmographic techniques may be employed for registering the arterial pulsations, but they are not so simple and convenient to use as is the separate registration cuff method.

When estimating the blood pressure by the auscultatory method, several conditions exist which may introduce error. As a general rule, the systolic pressure is underestimated, because the first Korotkow sound which appears when the cuff pressure is gradually lowered from above the systolic level possesses an intensity below the threshold of human audibility. The auscultatory method is based upon the supposition that the systolic blood pressure level is determined when the artery opens momentarily at the peak of the systolic wave and allows a small quantity of blood to pass below the pressure cuff. The mechanical and hemodynamic factors responsible for the Korotkow sound at this instant are of low magnitude. Thus, the sound level of the first Korotkow sound must be correspondingly low in intensity, and it so happens that in a majority of human beings this intensity is below the threshold of human hearing. Under such conditions, the first audible sound cannot always be considered as representing the level of systolic blood pressure. Yet, this is common clinical procedure. Furthermore, the threshold of human hearing is variable and is dependent upon such factors as:

1. The efficiency of the observers hearing.
2. The efficiency of the stethoscope.
3. The type of stethoscopic chest piece employed.
4. The application pressure of the stethoscopic chest piece.
5. The general noise level in the room.
6. Auscultatory experience.
7. The mental and physical state of the observer, especially with respect to fatigue at the time the reading is taken.

We have observed, that as a general rule, the first audible Korotkow sound (heard by a competent observer under optimum conditions) is approximately 5 mm. of Hg below the first actual Korotkow sound that appears; rarely is the differential more than 10 mm. of Hg. Under less favorable conditions, the differential may be considerably greater.

The diastolic pressure as judged by the Korotkow method (when the sounds suddenly become dull and muffled) corresponds exactly with the recorded values and within close limits as compared with the negative transition effect of the arterial pulsation. *Any other sound phase does not bear any relationship whatsoever with diastolic pressure.*

An interesting observation in the registration of the Korotkow sounds is that the five phases are registered by both stethoscopic[56] and logarithmic[62, 63] phonocardiography. However, the stethoscopic method indicates the first Korotkow sound (systolic level) most distinctly, whereas the logarithmic method shows the sudden dull and muffled phase (diastolic level) more distinctly. The murmur components of the Korotkow sounds are best registered by the logarithmic method.

The effects that have been observed by von Recklinghausen, Gumprecht, Müller and Blauel, Janeway, and Ragan and Bordley with respect to the width of the pressure cuff correspond with our observations.

SUMMARY AND CONCLUSIONS

1. An apparatus was devised which can register graphically and simultaneously all of the physiologic phenomena which must be evaluated for the estimation of blood pressure by the palpatory, oscillatory, and auscultatory methods. This graphic device eliminates the human element with its subjective differences in the appreciation of the physiologic signs and makes possible a quantitative comparison of the associated phenomena.

2. The sphygmomanometric device used in this investigation is described.

3. The theoretical principles fundamental to the palpatory, oscillatory, and the auscultatory methods are discussed.

4. Following is our summary and conclusions on the *oscillatory blood pressure method:*

A. In an oscillometric curve, the configuration of the pulse wave is modified between the limits of systolic and diastolic pressure. The degree of modification is a function of the systolic, diastolic, and cuff pressures. The wave form is unmodified only when the cuff pressure is lower than the diastolic pressure level.

B. Diastolic pressure is represented in the oscillometric curve by the first arterial pulsation which is undistorted during its most negative phase.

C. The negative transition effect is rarely observable via the visual oscillometric method.

D. The resonance effect which occurs in most oscillometric registrations corresponds to the level of diastolic pressure. Although this resonance effect is observable in most persons, it is much too broad to be useful as an accurate indication of diastolic pressure.

E. The oscillatory method is reliable in the estimation of the systolic blood pressure only when accurate sphymographic registrations are made. Inaccurate or poor sphygmograms do not indicate distinctly the initial appearance of the minute wave which represents the beginning of ejection

of blood into the artery as differentiated from the supramaximal oscillations.

F. The gradual tapering-off of the pulse amplitude in an oscillometric curve below the diastolic pressure level commonly described and illustrated in the literature is not a physiologic phenomenon but a definite *instrumental error.* The arterial pulse does not gradually diminish and then disappear below the diastolic pressure level, but remains more or less of constant amplitude. The instrumental error is essentially due to *a diminution in the efficiency of the cuff as a detector of arterial pulsations* as the pressure is lowered. The use of independent pressure and registration cuffs eliminates this source of error.

5. Following is our summary and conclusions on the *Korotkow method:*

A. The Korotkow sounds occur simultaneously with the sharp primary oscillation of the brachial pulse which is the beginning of the ejection of blood into the artery.

B. There are two major contributing factors to the production of the Korotkow sounds; the mechanism of each is described.

C. The first distinct Korotkow sound (systolic pressure level) generally occurs simultaneously with the first distinct sharp primary oscillation of an oscillometric curve. The first suddenly diminished Korotkow sound (auscultatory diastolic pressure level) corresponds very closely to the first undistorted arterial pulsation.

D. An explanation is given for the sudden diminution in the intensity of the Korotkow sounds at diastolic pressure.

E. A secondary sound that is commonly registered, although seldom heard, occurs simultaneously with the incisura of the pulse curve. The mechanism of the production of this sound is similar to that of the Korotkow sounds.

F. When estimating blood pressure by the auscultatory method, seven major conditions exist which may alter the threshold of hearing and introduce error.

G. As a general rule, the systolic pressure is underestimated because the first Korotkow sound which appears during a gradual cuff deflation possesses an intensity below human audibility.

H. Diastolic blood pressure as judged by the auscultatory method (when the sounds suddenly become dull and muffled) corresponds exactly with the recorded values and within close limits with the negative transition effect of the oscillometric curve. *Any other sound phase does not bear any relationship whatsoever with diastolic pressure.*

I. The stethoscopic method of sound registration shows the first Korotkow sound (systolic pressure level) most distinctly, whereas the logarithmic method registers the sudden diminution (diastolic pressure level) more distinctly than the stethoscopic method.

J. The murmur components of the Korotkow sounds are best registered by the logarithmic method.

6. The effects that have been observed by von Recklinghausen, Gumprecht, Müller and Blauel, Janeway, and Ragan and Bordly with relation to the width of the pressure cuff correspond with our graphic data.

7. In some instances, the sudden muffling effect at the diastolic pressure level is not audible. In such cases, the graphic method may show this diminution effect with sufficient clarity to accurately estimate the diastolic blood pressure. We have never encountered a condition where the combination of auscultation, graphic registration of the Korotkow sounds, and the graphic oscillatory method did not distinctly indicate the diastolic pressure level; that is, at least one of the methods always gives a distinct and clear indication.

8. Considerable error may be introduced when estimating the systolic blood pressure by the palpatory method. The degree of error may be in the order of several millimeters of mercury below the actual value, because the initial primary oscillation is below the threshold of human feeling.

9. The diastolic pressure level cannot be estimated by the palpatory method, because the sense of human feeling cannot differentiate the point of transition where the negative flattening effect becomes peaked. The negative transition effect is the only reliable oscillometric method for the estimation of diastolic blood pressure.

10. The graphic registration method we have described, which registers all of the physiologic phenomena associated with indirect sphygmomanometry, has application in the more exact studies of blood pressure. The method shall prove useful in the clarification of such phenomena as the auscultatory gap, the double tone of Traube, the double murmur of Duroziez, the murmur accompanying the pistol shot pulse, etc.

We wish to express our appreciation for the cooperation of the Sanborn Company, of Cambridge, Massachusetts, in this investigation.

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Erratum

In the article by Rappaport and Rappaport entitled "An Improvement of the Serologic Kahn Reaction in the Spinal Fluid" in the August, 1943, issue of the JOURNAL, the procedure (second line from the bottom of page 1355) should read as follows:

> Pour 0.1 c.c. of serum (reagent II) in each of two small test tubes and inactivate it at 56° C. for twenty minutes, simultaneously with a third tube containing a little more than 1.5 c.c. of spinal fluid.

APPARATUS FOR THE FREEZING-DRYING OF TISSUES FOR STORAGE*

A. Cecil Taylor, Ph.D., Chicago

FREEZING-DRYING has been employed successfully as a method for preserving segments of nerve and artery to be used later as grafts in experimental animals.[1, 2]

In order to reduce to a minimum distortion of the cellular structure of tissues subjected to freezing and drying, as well as chemical changes during the subsequent period of storage, three conditions are of prime importance: (a) To freeze the whole tissue rapidly, (b) to dehydrate as completely as possible, and (c) to maintain a low temperature during the dehydration process. Aside from appropriate beakers and thermos flasks, no special equipment is needed for the quick freezing of tissues. However, the subsequent process of dehydration in high vacuum at a constant low temperature requires special apparatus.

Based upon the principles of the equipment used by Bensley and Gersh[3] and Hoerr,[4] a freezing-drying unit was built by the author embodying certain simplifications and alterations of design which adapt it for the particular use intended. Aside from the low cost and simplicity of the apparatus here described, its chief advantage for our use lies in the fact that the tubes in which tissues are desiccated are easily accessible and can be sealed off without breaking the original vacuum. The dried tissues are thus protected from any contact with atmospheric air and moisture.

The apparatus consists of two systems, a vacuum desiccation system and a cooling system.

THE DESICCATION SYSTEM

The vacuum for desiccation is maintained by a Cenco high-vac pump. This has proved satisfactory when used alone, although the addition of a mercury diffusion pump in series would shorten the time required for drying the tissues at the low temperature necessary.

The pump (*A*, Fig. 1) is connected directly with a manifold (*B*) constructed of 2-inch galvanized iron pipe and fittings. The manifold is furnished with 9 nipples (*D*) for the attachment of the vials containing the tissues, and one (*E*) which may serve as an air inlet or as a place for the attachment of a vacuum gauge. All threaded joints were carefully sealed to insure against leakage. Since the movement of gases at very low pressures is greatly retarded by long, small caliber channels, the vacuum system was designed with the largest feasable inside diameters and shortest possible distances with the elimination of "bottle necks."

*From the Department of Zoology, The University of Chicago.

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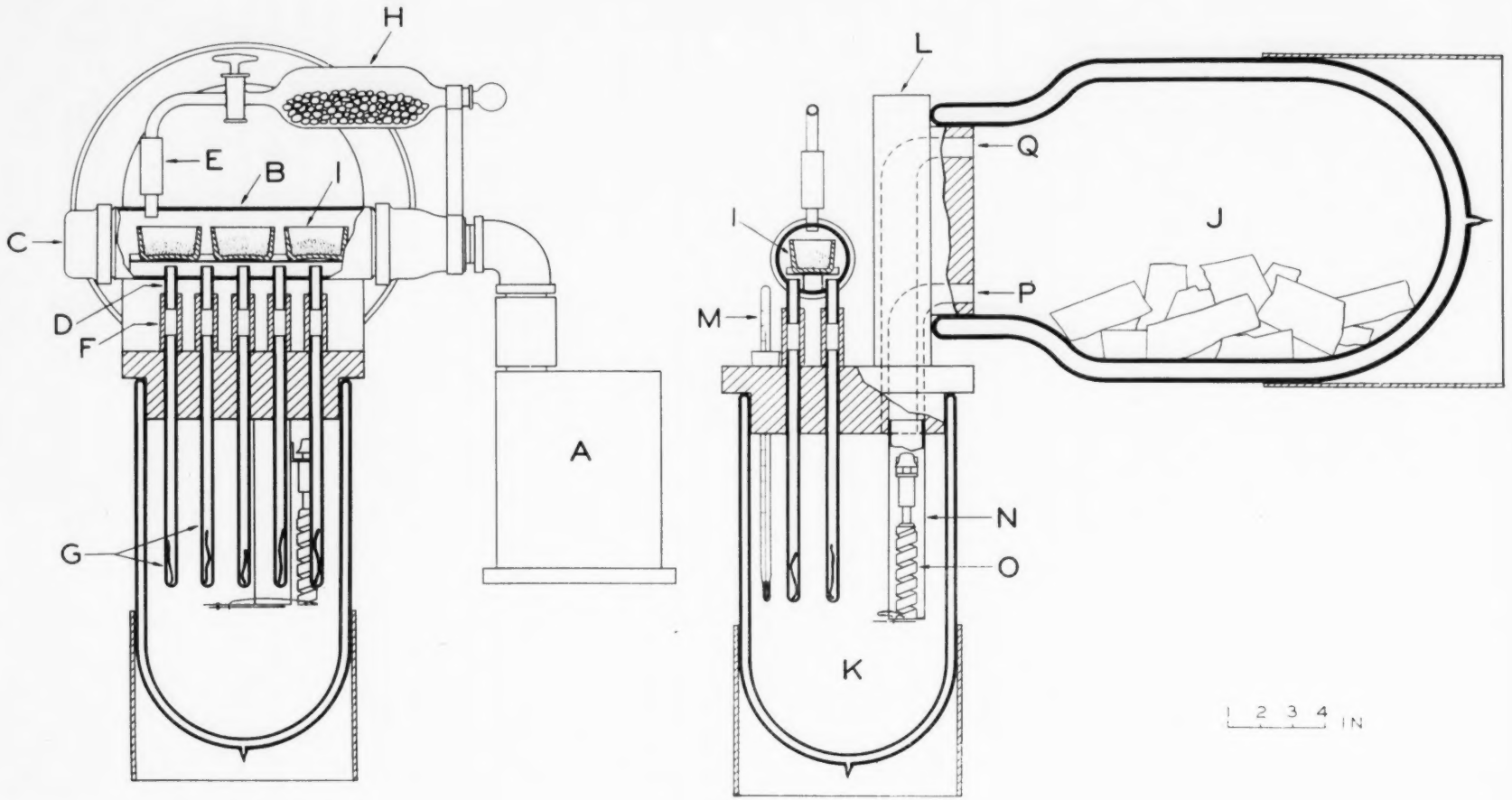

Fig. 1.

To protect the pump from moisture and to speed desiccation of the tissue, phosphorus pentoxide is placed in the manifold in dishes (*I*) set in a tray which can be removed from the manifold after unscrewing the manifold cap (*C*). Hermetic seal of this end of the manifold is insured by inserting into the screw cap a closely fitting steel disk and a rubber gasket.

Unless a gauge is to be used in the vacuum system, the extra nipple (*E*) is connected with a calcium chloride air dryer (*H*) controlled by glass stopcocks. This serves as an air inlet whenever it becomes necessary to break the vacuum before sealing off the tissue vials, e.g., in recharging the manifold with fresh phosphorus pentoxide during a run.

The frozen tissues are dehydrated in vials 20 cm. long, made from pieces of 10 mm. stock pyrex tubing by sealing one end in a flame. Nine of these tissue vials (*G*) may be attached to the manifold at one time by means of rubber pressure hose connections (*F*).

THE COOLING SYSTEM

In the preservation of bacteria suspended in liquid, or of fluids such as plasma, the cooling effect due to the evaporation within the evacuated tubes is sometimes depended on to maintain the material in a frozen state.[5] However, in the case of dense tissues such as nerves, with relatively low water content, evaporation is not sufficiently rapid to keep the temperature securely below the freezing point of the protoplasm and intercellular fluids. Especially is this true as dehydration progresses and the electrolytes of the tissue become more concentrated with a consequent lowering of their freezing point. For such tissues the highest temperature at which dehydration may be carried on without too rapid growth of ice crystals is in the vicinity of −40° C.[6]

In the more elaborate systems (Bensley), cooling is effected by mechanical refrigeration units with coils in heavily insulated chambers. These have the advantage of being reliable and accurately adjustable, but are cumbersome and expensive. In the apparatus here described a device for the use of solid carbon dioxide (dry ice) as a cooling agent was constructed. It consists of two thermos flasks connected by convection vents. One flask serves as source of cold gas which is utilized in the other for the refrigeration of the tissues. The gases in the former are kept near the sublimation temperature of carbon dioxide by packing the flask with dry ice, while the temperature in the latter may be maintained at the desired level by controlling the convection currents between the two flasks. The dry ice chamber (*J*) is a 12 liter Pyrex thermos flask laid on its side. A 4½ liter Pyrex thermos flask with wide mouth provides the constant temperature chamber (*K*) for refrigerating the tissues. Both flasks are closed by a common cover unit (*L*) which contains the convection vents (*P*, *Q*) as well as perforations for the tissue vials. The cover unit, details of which are shown in Figs. 1 and 2, is made of several thicknesses of ½ inch celotex stock cut to shape, assembled, and held together with large wood screws. This laminated construction provides a simple method of fashioning the gas vents. Several coats of shellac over the outside of the cover unit prevent the seepage of moisture into the system through the pores of the celotex.

The cold gas vent (*P*) is extended downward into the refrigeration chamber (K) by a metal tube (*N*) attached to the cover unit. This bears at its lower end

a thin metal damper valve (*S*, Fig. 2) which is adjusted to swing freely on its pivot so as to close off the vent. The damper valve is activated by a bimetallic thermostat (*O*) to which it is connected by a lever (*R*). By turning a knob (*T*) the thermostat may be adjusted to close the valve at any chosen temperature. Care was taken to provide a large cross-sectional area (not less than 5 sq. cm.) for the vents throughout their lengths in order to facilitate rapid convection currents. To permit the insertion of the tissue vials into the refrigeration chamber (*K*), holes were drilled into its cover, an extra hole being provided for the thermometer (*M*).

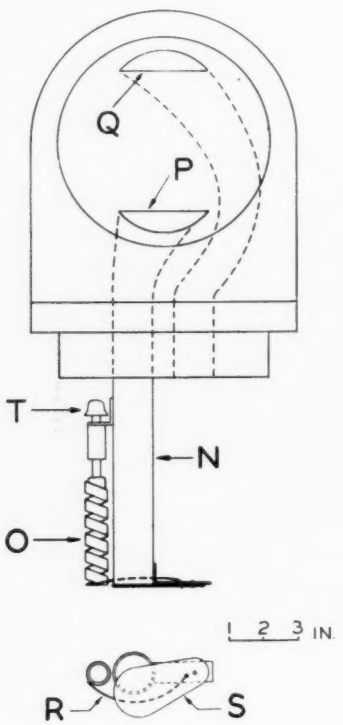

Fig. 2.

In operation, the cold gas mixture (air and carbon dioxide) within chamber *J* passes through the cold gas vent (*P*) and past the open damper valve (*S*) into chamber *K* where it displaces the lighter, warmer gas it encounters there. This rises to the top of the chamber and is forced through the warm gas vent (*Q*) back into chamber *J* where it becomes cooled by contact with the dry ice. As the gas in chamber *K* is cooled, the bimetallic helix is distorted so as to gradually close the damper valve and retard the convection current until an equilibrium is established at the temperature for which the regulator has been set.

A simple wooden framework provides support for both the desiccation and cooling systems (Fig. 3). The pump with its motor and the manifold are rigidly secured to the frame, while the thermos flasks and cover unit are demountable. In assembling these latter the cover unit is raised into the position indicated in the photograph after inserting the tissue vials into the holes provided for them.

The refrigeration flask (*K*) is then brought up over the tissue vials and fitted to its cover. It is supported in this position by drawing under it a sliding shelf. The dry ice flask (*J*) is cradled between the two horizontal members of the frame so that it may be slid onto its cover or pulled back at will.

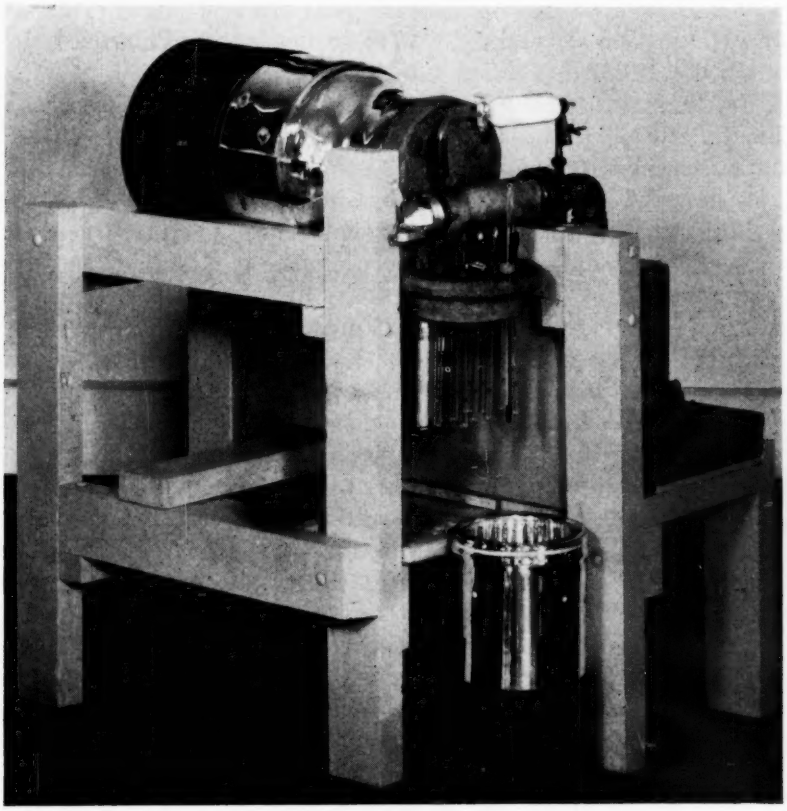

Fig. 3.

OPERATION

Tissues, having been prepared aseptically, are dropped directly into isopentane (practical grade, Eastman Kodak Co.) previously chilled in liquid nitrogen to a temperature of −150° C. About ten seconds is allowed for complete freezing of the tissue. Since it is important that the temperature of the frozen tissue be kept below −40° C., until it has become completely dry, precautions against warming must be observed at each step in the process of transferring the tissue from the isopentane into the assembled cooling chamber. Before removing the frozen tissues from the isopentane, the sterile Pyrex vials are cooled with dry ice. With chilled forceps, the frozen tissues are then dropped into the vials which may be loosely plugged with sterile cotton. After all vials have been filled, they are attached to the rubber couplings on the manifold nipples. This process must be done quickly, handling the cold vials with leather gloves cooled by holding a piece of dry ice for a short time. In order to keep already attached vials cool while others are still being handled, a container of alcohol charged with

dry ice is brought up under the vials until they dip into the liquid. When all vials are attached, the cover unit is raised into position over them, and the refrigeration flask (*K*), which has previously been thoroughly cooled with dry ice, is brought up onto its cover. Flask *J*, filled with dry ice, is now slid into position on its cover. The convection currents begin immediately. The desiccant dishes (*I*) are now filled with phosphorus pentoxide and placed in the manifold whose cap (*C*) is then screwed tight. The vacuum pump is started and evacuation continued until the desiccation of the tissue is completed.

The dry ice flask (*J*) holds between 15 and 20 pounds of solid carbon dioxide when filled to capacity. This amount maintains a temperature of −40° C., in the refrigeration chamber (*K*) for about 72 hours. During a long run 10 pounds of dry ice is added every second day by simply sliding flask *J* back from its cover, refilling with dry ice, and returning it to its cover again. One must guard against clogging the cold gas vent in the cover with pieces of dry ice during this refilling process.

The amount of phosphorus pentoxide necessary for a given run depends on the amount of moisture in the tissue to be desiccated. When all vials are heavily charged with moist tissue it has sometimes been necessary to replace the phosphorus pentoxide with fresh powder. To do this the vacuum must first be broken by opening the cocks in the air inlet desiccator (*H*) so as to admit air into the manifold very gradually. After air ceases to enter through the cocks, the cap *C* may be unscrewed and the desiccant dishes removed for refilling.

Most of the moisture extractable by the dehydrator is removed from the tissues during the first 48 hours; however, tissues to be stored for long periods have routinely been allowed to run from two to four days longer. The larger the pieces of tissue, the longer is the time required for drying. Tests with anhydrous copper sulfate show that nerves dehydrated for eight days still contain traces of water. However, it has been shown that, even after much more rigorous freezing-drying procedure, there still remains as much as 1 per cent moisture in the tissue.[4, 6]

The temperature within the refrigeration chamber (*K*) may be regulated by adjusting the thermostat controlling the damper valve. The degree of temperature constancy depends on the sensitivity of the thermostat and damper valve. Variations of no more than ±2° C. are characteristic of the equipment in use in our laboratory, which is entirely adequate for this purpose. If moisture can enter the cooling system through the pores of the cover unit or through improper fit of the covers, snow may form in the vents, thus cutting down, or shutting off completely, the convection current. Should this occur, the snow may be jarred loose by tapping on the metal tube (*N*) or by inserting a rod into the vent from below after removing flask *K*. When the dry ice in chamber *J* is reduced to less than 5 pounds, the temperature of the system begins to rise.

It should be noted that there is a temperature gradient from the bottom to the top of the refrigeration chamber (*K*). Therefore, to determine the temperature of the tissues in the vials, the bulb of the thermometer (*M*) should be on a level with the bottom of the tissue vials.

On completion of dehydration, the vials containing the tissue may be sealed off, either after filling with air through the desiccator (*H*), or while still evacuated. The cooling chambers and cover unit must first be removed. Then with

one vial at a time insulated from the rest by an asbestos sheet, the flame of an oxygen hand torch is played upon the walls of the vial at a point somewhat above the middle. When the glass is softened, the lower part which contains the tissue is drawn away and sealed. If the tissue is to be sealed in vacuum, the pump is left running while each vial, in turn, is shut off from the vacuum manifold by a screw clamp on the rubber connection at *F*. This is done as a precaution against the entrance of moist air into the whole system in the case of accidental perforation of the wall of the vial. Care must be exercised while sealing not to overheat the glass at any point, since, with tubing of this diameter, atmospheric pressure may easily force an opening through the softened glass.

Tissues sufficiently dehydrated and thus sealed in vacuo may then be stored at room temperature.

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A MECHANICAL IODINE APPLICATOR*

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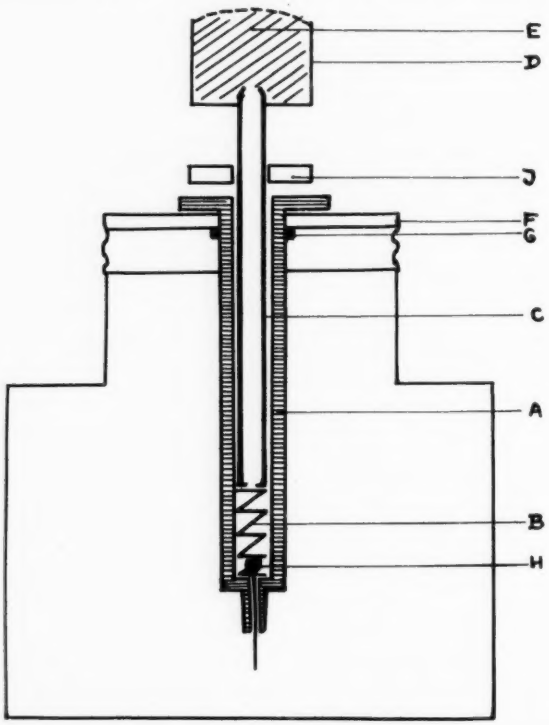
IN NUMEROUS laboratories large series of mice are often injected intra-abdominally, e.g., in routine mouse protective tests for the assay of antisera. Usually tincture of iodine, or some other liquid disinfectant, is applied to the skin of the abdomen of the mouse before the abdomen is punctured. This has the purpose of preventing the introduction of organisms present on the skin, into the peritoneal cavity, and also of killing pathogenic organisms that may



accidentally escape from the puncture wound. Commonly the disinfectant is applied with a cotton swab dipped into a bottle containing the disinfectant, after each application. Such a technique makes it necessary to deposit the syringe after the injection of each animal in order to take up the iodine applicator, and therefore usually involves considerable loss of time. To avoid such inconvenience, a mechanical iodine applicator, which has been time-saving in our hands, was constructed. The important feature of the mechanism is a small pump constructed as follows (Fig. 1):

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Into the bottom of the barrel (*A*) of an ordinary 2 c.c. syringe a soft spring (*B*) is inserted to hold up the plunger (*C*), which has been transformed into an open tube by removing the upper and lower ends. At the upper end of this tube a vaccine-bottle rubber stopper, from which the center has been cut out, is slipped on to serve as container (*D*) for firmly packed cotton and gauze (*E*) which serves as applicator pad. The barrel (*A*) of the syringe is held firmly in the screw top (*F*) by means of the rubber band (*G*). To serve as ball valve, an ordinary steel pin (*H*) with round head is dropped into the bottom of the barrel. The amount of liquid forced up into the container (*D*) at each stroke of the pump can be adjusted by the interposition of rubber washers (*J*) between the bottom of the container and the top of the barrel.

Before starting to inject a series of mice, the Pad (*E*) is pressed down repeatedly until it is soaked with disinfectant. The operator then takes the filled syringe into one hand, picks up a mouse with the other, presses the animal's abdomen gently against the pad (a pressure of ca. 20 Gm. is sufficient), which in turn is pushed downward, and pumps liquid up. The brief gentle touch produces an even round moist spot of disinfectant on the skin of the animal through which the injection is made. The operator then relinquishes that animal, picks up another one, and proceeds similarly without depositing the syringe before it is empty.

Summary.—A simple mechanism is described which permits the rapid automatic application of a graded quantity of liquid disinfectant to the abdomen of mice injected intraperitoneally in large series, and which results in considerable saving of time.

CHEMICAL

NOTE ON THE SELENIUM METHOD FOR THE DETERMINATION OF NONPROTEIN NITROGEN*

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THE principal advantage of the method of Reis and Powers[1] for the determination of nonprotein nitrogen in blood is that the digestion tubes do not become etched. The necessity for conserving apparatus makes this feature of value at the present time. Since the publication of the method, several points that need clarification have come to our attention and are set forth in this note.

1. While the selenium-sulfuric digestion mixture prepared from selenious acid gives no trouble, the one prepared by dissolving selenium in sulfuric acid and diluting with water often develops a red amorphous precipitate of selenium. This is caused by insufficient cooling during the addition of the water. While this precipitate does not render the reagent unusable, we now suggest the following method of preparation in which there is little danger that a precipitate will form.

Dissolve with heat 70 mg. of selenium in 10 c.c. of concentrated sulfuric acid. As the selenium dissolves the solution will become first green and then clear with a slight yellowish cast. Cool thoroughly and add 200 c.c. of cold 40 per cent sulfuric acid.

2. The instructions read to have the flame of the microburner 1 inch high and the bottom of the Pyrex tube ¾ inch above the top of the burner. This is commonly interpreted to mean ¾ inch above the top of the flame, whereas it should actually be ¼ inch in the flame. With this adjustment the boiling will be vigorous and continuous, and bumping will not be as likely to occur.

3. In the Folin-Wu method it is common practice to disregard very slight turbidities, but in this method a turbidity that is scarcely visible to the naked eye may introduce a considerable error. However, no turbidity will occur if the tube is thoroughly cooled after digestion, and all subsequent steps in the nesslerization are completed as rapidly as possible. If properly done, the nesslerized solutions may be allowed to stand several hours before reading, with no development of turbidity.

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*From the Department of Biological Chemistry, Tufts College Medical School.

MEDICAL ILLUSTRATION

FACIAL AND BODY PROSTHESIS IN RELATION TO WAR WOUNDS

CAPT. CARL DAME CLARKE, A.U.S.

EVERY war has produced wounds that require both plastic surgical repairs and prosthetic appliances. The advent of high explosives and inflammable mixtures and the extensive use of the airplane have increased greatly the necessity for such work. Although plastic surgery is a relatively new field in comparison with other branches of medicine, the really worth-while advances in prosthetic appliances have been made only in the last twenty years. For this reason, the average physician or surgeon may not ascribe to them the importance they rightly deserve. Often the surgeon considers only plastic surgery, when he has at his disposal a method of repair that is high in cosmetic, psychologic, and physical value.

The purpose of this paper is to bring facial and body prosthetics to the surgeon's attention so that patients may benefit from the possibilities.

The technique of producing these prosthetic appliances has been described previously in considerable detail by Clarke,[1-8]; by Bulbulian,[9, 10] and by Kazanjian, Rowe, and Young.[11] Therefore, this part of the subject will not be considered here.

Facial and body prostheses are divided into two main groups as follows:

1. Those that are purely of a temporary nature, for cosmetic purposes only, provided to be worn until the defects can be corrected permanently by plastic surgery. Typical examples are cases in which an artificial tip of a nose or a section of a cheek are made to replace temporarily the actual parts lost through disease or injury. In such instances plastic surgery will be done later and will obviate the necessity for the prostheses.

2. The second group is comprised of cases beyond the help of plastic surgery. A typical example of this is an artificial hand made to replace a missing natural hand. The artificial hand at present available should not be confused with the prosthesis hitherto employed. Formerly the hand was carved from wood or constructed from metal and then covered with a leather glove. It looked unnatural at all times. Now the prosthetic hand is made of rubber, with the shade and texture corresponding to that of a real hand and containing all the skin detail. In fact, a fingerprint impression or pattern can be made as easily from an artificial rubber hand as from a natural hand. Since the rubber is filled with a plastic material which allows the fingers to be bent into any position, it may be used for writing, eating, or for carrying light objects. It, too, can be covered with a leather glove when desirable; however, this is unnecessary because the rubber hand has the same color and texture as the natural one.

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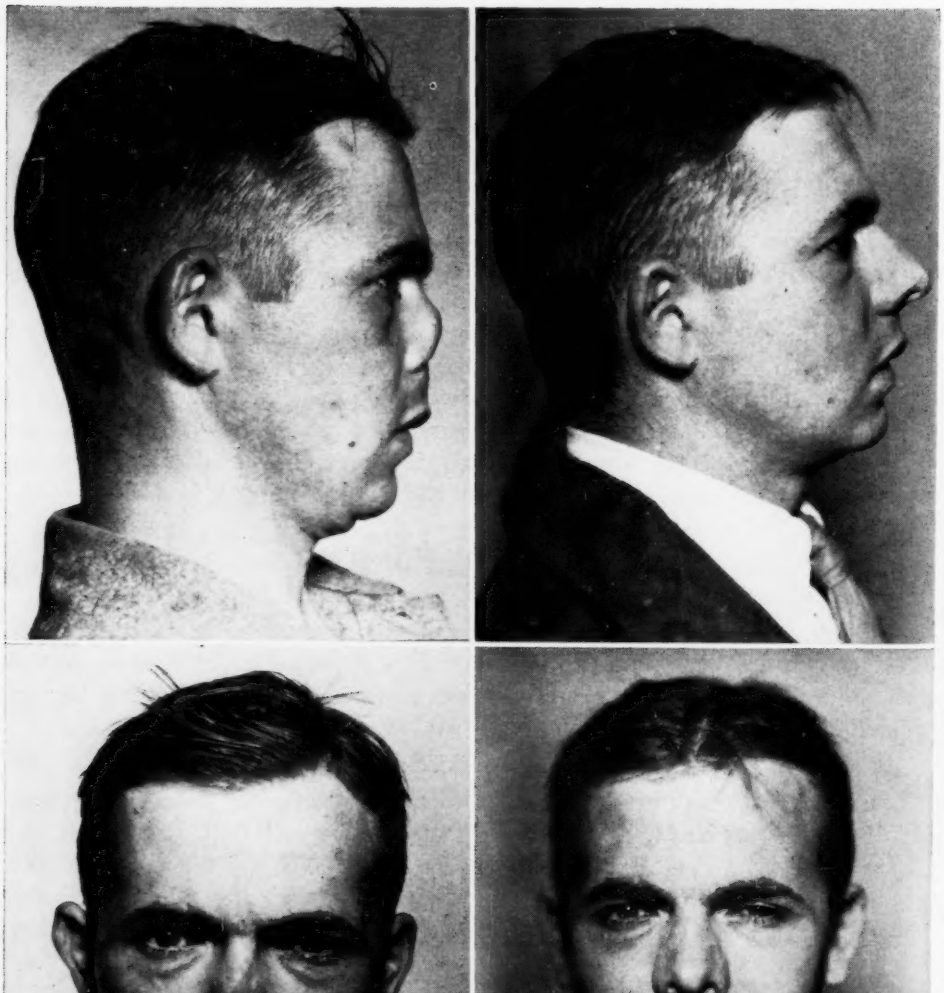

Fig. 1.—Top, profile view of a patient before and during the wearing of a prosthesis of a nose. Bottom, a front view of the same patient.

At times the surgeon and prosthetist together should decide which method of repair, surgical or prosthetic, will be most beneficial to the patient. One method alone should not be considered in all cases. In other words, the surgeon and prosthetist should weigh the value, desirability, and debilitating effect to the patient both physically and psychologically. When an entire ear has been destroyed, it may be considered advisable to replace the missing organ with a soft rubber prosthesis, rather than to attempt the reconstruction of the ear by plastic surgery. Such surgical procedure has not, to date, been entirely successful. When it has been attempted, numerous operations have been performed over a period of years. In fact, one exceptionally competent surgeon performed an average of three minor operations a year over a period of fifteen years to reconstruct an ear. It is possible that methods will be perfected in

Fig. 2.

Fig. 3.

Fig. 2.—A profile view of the same patient as seen in Fig. 1. The eyeglasses are used to help hide the join lines where the prosthesis comes into contact with the skin. An adhesive is employed to hold the prosthesis in place.

Fig. 3.—A front view of the same patient, as seen in Figs. 1 and 2, with eyeglasses in place. Dark lenses are unnecessary. In fact, the frames alone are suitable. The prosthesis is not attached in any way to the frames.

the future which will make the prosthetic ear superfluous. The same criticism applies to the reconstruction of the nose when the entire organ has been destroyed. However, if both natural alae remain, a presentable nose may be reconstructed by plastic surgery. Although the surgical procedure may be lengthy and painful to the patient, with considerable detrimental psychologic effect, nevertheless the surgical repair becomes a permanent part of the body. It is also logical to reason that should a patient have a minor defect which can easily be corrected by plastic surgery, time and expense should not be consumed in preparing a temporary prosthesis.

In comparison to lengthy surgical procedures a prosthesis requires but a few hours of time to make. It causes the patient no inconvenience or pain during the preparation, application, and wearing. However, it always remains

Fig. 4.—A prosthetic hand. The patient's left hand (the hand on the right in the photographs) is artificial. It is filled with a plastic material which permits the fingers to be bent into desired positions for use.

an artificial part which must be removed, cleaned, and replaced from time to time.

A face prosthesis, if properly compounded and technically executed, will last for years. A prosthetic hand receives more wear and for this reason must be replaced more often. This is of little consequence because at any time, a new hand can be made from the original plaster or metal mold, which is filed away for this purpose. In fact, under favorable conditions, artificial rubber hands can be supplied immediately from stock molds to replace those members

lost by disease or accident. Invariably one of these will be found to suit the patient.

Under no condition should a temporary or permanent prosthesis be made until the normal tissues have healed completely and all evidence of edema and swelling have disappeared; otherwise the final prosthesis will not fit the remaining parts of the body. *Join lines*, or lines formed where the artificial part joins the natural tissues, will become too evident. Cosmetics, make-up putty, and eyeglass frames cannot be relied upon to hide these join lines if the prosthesis does not fit properly. The failure of a prosthesis usually is caused by faulty technique on the part of the prosthetist or by the fact that the mold was made before the swelling and edema subsided. In the latter case the prosthesis will not fit after the tissues have changed in size. When a prosthesis is made to fit over a hairy surface, such as the upper lip or lower jaw, the hair must be kept shaved from the skin where the prosthesis is to adhere to the surface. Otherwise, the hair will force the prosthesis from the surface and result in poor adhesion and visible join lines.

Fig. 5.

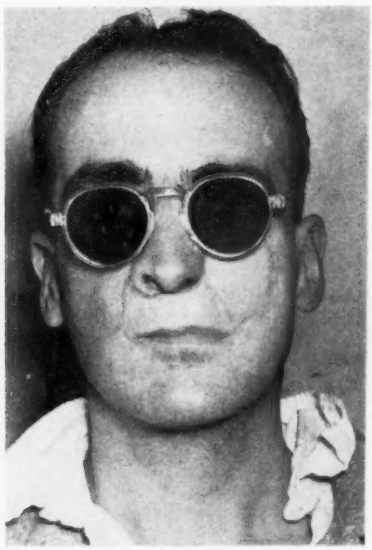

Fig. 6.

Fig. 5.—A photograph of a patient without a prosthesis.

Fig. 6.—The same patient as seen in Fig. 5 with a temporary prosthesis in place. A prosthesis of this type is made for psychologic and cosmetic purposes only, until the necessary plastic surgery can be accomplished. In this instance dark glasses were used to hide the eye socket until an artificial eye could be supplied.

When a good adhesive of the proper consistency is used, the patient need have no fear of losing the artificial part; it will cling firmly to the surface. If the prosthesis is removed from time to time and cleaned before it is replaced, there will be little possibility of infection. In fact, no case of irritation or infection from the wearing of a prosthesis has yet been reported. There have been recorded two cases of patients having an idiosyncrasy to the gum mastic used in the adhesive. This difficulty could have been overcome by substituting another gum, such as colophony, which serves equally well. The author has made prostheses for physicians themselves; and, after years of use, they have

reported that no infections or irritations ever developed. One of these physicians removed his prosthesis every night. Another wore his for a period of fourteen days before removing it for cleaning.

The face and hands may be washed while the prosthesis is in place without danger of it coming off or of drastically changing the esthetic effect. However, it is desirable that cosmetics be applied after washing. Finally, it must be realized that a well-compounded prosthesis will not change in color or shade. This does not hold true in regard to skin tones. In summer the skin darkens; in winter it lightens. The patient should therefore be supplied with three prostheses of slightly different colors to allow for the changes of the normal skin. This necessitates but little additional work on the part of the prosthetist, for many rubber casts can be made from the same plaster mold in a short period of time. The application of a lotion or powder base and face powder compensates for the slight color changes that may exist between the actual skin and the prosthesis.

Prosthetic work in the past has been done primarily by dentists, and invariably this has applied to the oral cavity proper. However, some cases, such as wounds of the lower jaw, should have temporary skin prostheses made to prevent the continuous dribbling of saliva until a plastic repair may be accomplished. As soon as the loss of saliva is checked, the patient feels better and gains weight. In turn, this speeds the day on which a plastic procedure may be followed to effect a permanent repair. It must be reasoned that saliva is essential to normal gastronomic functions and its loss impairs the general health of the patient.

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